BASIC MEDICAL MICROBIOLOGY

ROBERT F. BOYD, Ph.D.
BRYAN G. HOERL, Ph.D.

THIRD EDITION

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MV

To Beverly, Daniel, Diana, Robin, and Timothy

R. F. B.

To my wife Pat, and to my children Bryan, Janet, Michael, and Daniel, and their spouses

B. G. H.

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PREFACE

A significant number of discoveries and innovations have occurred in microbiology since the first edition of this book was published in 1977. It was our intention at that time to present a book that provided up-to-date and formalized coverage of medical microbiology with less emphasis on general microbiology. Times change, however; science advances and textbooks must alter their directions accordingly. While we have not changed our emphasis on medical microbiology in the third edition, we have broadened our coverage of some aspects of general microbiology to keep pace with more recent discoveries in that field, many of which have been in the areas of genetics and molecular biology. Some teachers of allied health programs would argue that their students do not need this information, but we believe that since microbiology and its associated sciences are influencing and will continue to influence the quality of our lives either directly or indirectly, we can no longer ignore the impact of biochemistry and molecular biology on the study of any branch of microbiology. To teach microbiology merely as a descriptive medical science is to ignore some of the most exciting and far-reaching discoveries that have been made in modern biochemistry and molecular biology. However, we have kept the discussions in these areas to a minimum to prevent the reader from getting bogged down with unnecessary details. In addition, we have significantly increased the number of illustrations to better explain the more difficult concepts within these new chapters. To make the relevance of biochemistry to microbiology clearer to the student, an introductory chapter called Biochemistry of Macromolecules has been added to this edition, and the discussion of enzymes, which was a separate chapter in the second edition, has been incorporated into it.

While the second edition used the section on General Microbiology to discuss prokaryotic (bacterial) structure and function, exclusively, the third edition has one chapter devoted to the general characteristics of prokaryotes and eukaryotes with more information on eukaryotes than was included in previous editions. The major discussions of the general characteristics of eukaryotic infectious agents, such as fungi and protozoa as well as the viruses, are still reserved for later chapters on in-

fectious disease. The chapter on the isolation, cultivation, and identification of bacteria in the second edition has been broken up; cultivation and isolation are now combined with nutrition in a single chapter, and identification appears later in Chapter 18. Overall, the section on general microbiology has been enlarged, but some of the specific topics can be eliminated by the teacher without breaking the continuity of any chapter.

Section III, Host-Parasite Interaction, has been expanded to include a separate chapter on epidemiology. The two chapters in this section are probably the most important in terms of grasping the basics of the infectious disease process and, if thoroughly understood, make future chapters easier to comprehend.

Because of recent advances in immunology, section IV includes several new additions and interpretations. Described for the first time are the mechanisms of recombination in somatic cells that account for antibody diversity; the heavy chain switches that affect the biological functions of an immunoglobulin molecule; the roles of the major histocompatibility complex genes that code for cell surface glycoproteins (which are important to antigen recognition and to interaction among immune cells); the hypothesized roles of idiotypes and anti-idiotypes in the regulation of the immune system; and the methods of production of monoclonal antibodies in more rapid and precise immunodiagnosis and in immunotherapy.

Section V, Bacteria That Cause Infectious Disease, has also been altered in several ways. Chapter 18, Introduction to the Bacterial Diseases, has been expanded to include the identification of bacteria as well as a description of basic terms and approaches to the study of bacterial diseases. Actinomycetes such as *Actinomyces* and *Nocardia,* which were included with the pathogenic fungi in the second edition because of their similarities in pathology, have been placed with their natural taxonomic group, the bacteria. *Legionella,* which was given a separate chapter in the second edition, is now included in Chapter 32, Miscellaneous Pathogens.

The remaining sections of the book, which include chapters on viruses, fungi, animal parasites, and selected topics, have all been revised and expanded. Throughout Sections V–VIII the reader will find greater discussions of the mechanisms of pathogenesis, including many new illustrations and tables that are sure to aid the student in comprehending the material. Special boxed essays illustrate pertinent aspects of the infectious disease process; all of these essays have been derived primarily from the *Morbidity and Mortality Weekly Report,* which is issued by the Centers for Disease Control in Atlanta, Georgia.

We hope that those who used the previous editions of this book as well as those using this edition for the first time will find the chapters and illustrations we have added to be of value.

No book is published without the help of many people. We would like to thank the reviewers of the second and third editions for their helpful comments and suggestions. A special thanks is needed for the many authors and publishers of books and journal articles who allowed us to use the tables and photomicrographs that appear in this edition. We are especially grateful to Will Kos of Marquette University School of Dentistry for his willingness to revise and update the chapter on oral microbiology. We thank Sue Seif and Dawn Braatz for the line drawings, whose clarity and originality will be of immense value to the student. We would also like to thank the typists, Beverly Boyd and Judy Williams, as well as the staff members of Little, Brown and Company, especially Barbara Ward, for their editorial assistance.

R. F. B.
B. G. H.

I. GENERAL MICROBIOLOGY

1. SCOPE AND HISTORY OF MICROBIOLOGY

OBJECTIVES

To understand the importance of microbiology to medicine as well as to applied areas of science

To describe the contributions of the following scientists to microbiology: Leeuwenhoek, Pasteur, Koch, Lister, Jenner, and Ehrlich

To explain what is meant by the theory of spontaneous generation and the experiments that were performed to refute it

To list the steps that are required to identify the causative agent of an infectious disease

Scope
History
- Early Concepts of Disease
- First Observations of Microorganisms
- Spontaneous Generation
- Germ Theory of Disease

The Golden Age of Microbiology (1870–1890)
- Isolation Techniques
- Staining
- Immunology

Virology
Chemotherapy
Modern Microbiology

SCOPE

Microbiology is a science that is primarily concerned with the study of microorganisms and viruses. A distinction between microorganisms and viruses is made because viruses do not exhibit cellular characteristics. Viruses are entities that are totally dependent on the presence of a living cell for replication. Microorganisms are for the most part single-celled, but they also include certain multicellular types. When we speak of microorganisms, we must include bacteria, fungi (molds and yeasts), certain algae, and protozoa. Protozoa belong to the group called *animal parasites*, which also includes certain multicellular worms callled *helminths*. Algae are not associated with disease in humans and will not be discussed in this text.

The explosion of microbiological information in recent years has been so great that it has resulted in the creation of specialties concerned with each of the representative groups of microorganisms being studied. For example, the bacteriologist may develop skills in various aspects of bacteriology such as bacterial physiology, bacterial genetics, or bacterial cytology. The microbiologist may specialize in the study of microorganisms as they relate to applied and environmental fields. There are now divisions of microbiology such as space microbiology, soil microbiology, aquatic microbiology, food microbiology, and industrial microbiology.

Medical microbiology, which deals primarily with microorganisms that cause infectious disease, is the branch of microbiology with which the student reading this book is primarily concerned. Since various representative types of microorganisms can cause disease in humans, the medical microbiologist must have a basic knowledge of the chemical and physical properties of the potentially harmful microbial agents—bacteria, viruses, yeasts, molds, and protozoa. The primary responsibility of the medical microbiologist is to understand the etiology (causation), disease manifestations, laboratory diagnosis, and treatment of infectious disease. The microbiologist's obligations may also include determining the epidemiology of disease (e.g., how diseases are transmitted) and developing measures for the control and prevention of diseases in the community. The fact that many major pestilences such as smallpox, diphtheria, and plague no longer decimate populations as they once did is testimony to the advances made in epidemiology, control, and prevention of infectious disease. Resistance or immunity to disease has been recognized for years. This area of study is referred to as *immunology* and deals specifically with the relationship of antigens or foreign substances to antibody production in the host. Because of its relationship to disease, immunology is also considered an important part of the medical microbiologist's background. The development of new concepts in molecular biology and cellular immunity has not only expanded our knowledge of the immune process but has also provided the microbiologist with new laboratory diagnostic tools. The medical microbiologist can now identify and classify microorganisms using immunological techniques.

In its early history, microbiology as a science was concerned with the identification and control of microorganisms. Major advances in microscopy

and biochemical techniques from 1940 to the present have enabled scientists to use microorganisms as a model for the study of biological properties, particularly in the areas of genetics and metabolism. These studies are aided by the fact that microorganisms divide very rapidly and are easily cultivated and maintained in the laboratory—properties not common to higher forms of life.

During the development of microbiology, it was soon realized that many of the metabolic processes occurring in microbial systems were similar if not identical to those of cells in higher systems, including the human. In 1944 Avery, MacLeod, and McCarty discovered that isolated (cell-free) DNA was capable of transforming (producing a genetic change in) certain intact bacterial cells. This was one of several experiments proving that DNA was the hereditary material of the cell. The discovery of the structure of DNA by Watson and Crick in 1953 and the results obtained from experiments in microbial genetics provided the basic clues to genetic mechanisms not only in bacteria but in higher forms of life as well. Scientists are now able to manipulate genes and to transfer genes from one species of organism into the genomes of totally unrelated microbial species. Such techniques referred to as *genetic engineering* are revolutionizing microbiology and related sciences. This technique, along with our greater understanding of molecular biology and biochemistry, is advancing us near the brink of conquering, or at the very least tempering, many of the major diseases, both infectious and noninfectious, that now afflict humankind. In addition, many biological properties unavailable to some organisms, such as plants' ability to fix nitrogen from the air or the capacity to produce large quantities of a single protein to be used as food, may become realities in the very near future. The future for microbiology, both medical and nonmedical, is so bright and promising that we can hardly wait for each day's new discoveries to be revealed. We hope that the importance of these discoveries will be imparted to you as you read each chapter. But keep in mind that you can fully appreciate these discoveries only if you understand the basic biological properties of microorganisms.

HISTORY

EARLY CONCEPTS OF DISEASE

Microbiology had its origins in the concepts that were first formulated to explain disease. In some ancient civilizations disease was believed to be a punishment sent from the gods for human wrongdoings. Many of the philosophers during these early periods in history, however, were of the belief that disease was transmitted by invisible "animals." Since the animals could not be seen, the theory remained just that, a theory. The Italian physician Fracastorius (1485–1553) later postulated that disease was transmitted by invisible particles or seeds from one person to another or from contact with the clothing or utensils of the infected. Two hundred years elapsed before a detailed description of microorganisms was made.

FIRST OBSERVATIONS OF MICROORGANISMS

Anton van Leeuwenhoek (c. 1685), who took up microbiology as a hobby, was an amateur microscope builder. Using a very primitive microscope, he described in some detail the structure of the red blood cells of humans and other animals. Leeuwenhoek was the first to describe microscopic organisms found in pond water and later made observations of bacteria he found in the debris surrounding teeth (Fig. 1-1). Leeuwenhoek's microscope was a little over 2 inches in length and capable of magnifications approaching 160 to 200 times (Fig. 1-2).

SPONTANEOUS GENERATION

Even though his was the first description of microorganisms, most of Leeuwenhoek's peers either ignored or denied the existence of these newly discovered organisms. Instead, most scientists accepted the theory of *spontaneous generation*, that is, that life can arise from dead organic matter—a theory first proposed by Aristotle in 384 B.C. For

Fig. 1-1. Shapes of microorganisms observed by Leeuwenhoek in samples taken from the human mouth. A. Rod-shaped (bacillus). B. Spherical (cocci). C. Spiral-shaped (spirochete). D. Cigar-shaped rods.

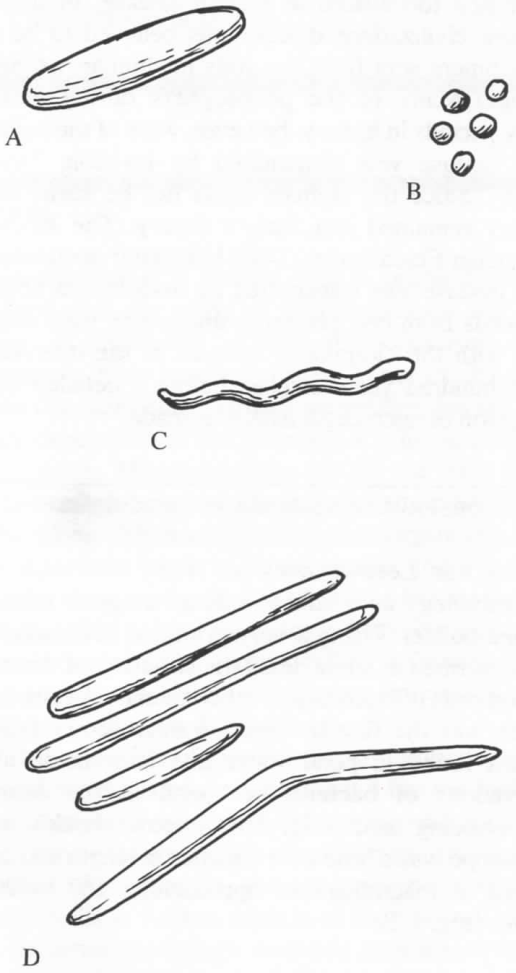
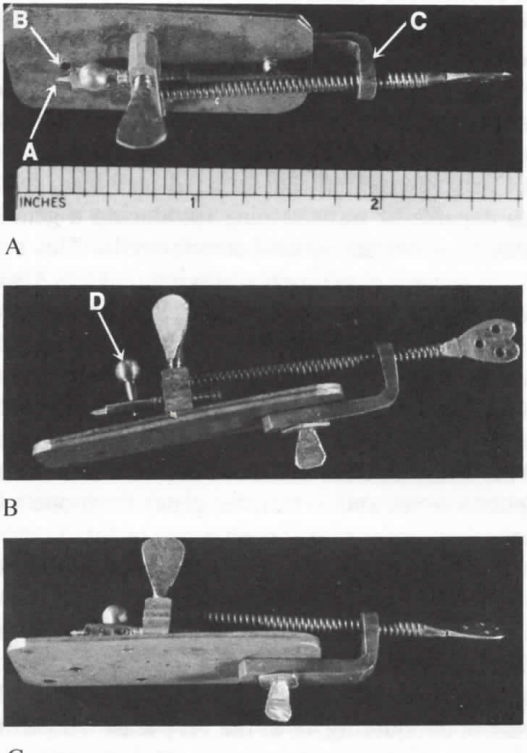


Fig. 1-2. Actual-size replica of Leeuwenhoek microscope from Leyden, Holland, showing various views of instrument. *A*, pin on which object is placed for viewing; *B*, lens; *C*, screw for coarse adjustment; *D*, screw for fine adjustment. (From D. A. Anderson and R. J. Sobieski, *Introduction to Microbiology* [2nd ed.]. St. Louis: Mosby, 1980.)



A

B

C

example, the scientific community was convinced that blowflies could arise spontaneously from rotted meat. Scientists such as Francisco Redi (1668) and Spallanzani (1776) performed experiments that showed that organic matter, if protected from contamination by boiling and preventing exposure to the air, did not give rise to new life. The spontaneous generation proponents refuted these experiments by suggesting that a vital force, such as air, had been destroyed by boiling.

Franz Schulze and Theodore Schwann in 1836 independently demonstrated that air was not the vital force. Schulze passed heated air into flasks of nutrient broth and showed that heating sterilized the air and prevented growth in the flasks. Schwann passed air through chemical solutions before it entered the flasks containing nutrient media and obtained the same results. The proponents of spontaneous generation were unswayed by these experiments. They countered that the treated air had been stripped of any life-generating forces. Despite these and other experiments that disproved spontaneous generation, the proponents of this theory were still unconvinced.

Only the experiments of Pasteur (Fig. 1-3) would finally put an end to the theory of spontaneous generation. Using swan-necked flasks (Fig. 1-4), Pasteur boiled organic solutions to destroy any "seeds" (microorganisms) that might be present. There was no barrier to the passage of air in these flasks, and they could sit for several days with no visible turbidity, which would indicate the presence of life (seeds or microorganisms). In other words, air outside the flask could diffuse into the organic broth, but any microorganism carried in the air could go no further than the walls of the flask's neck where it would settle out. If the liquid in the flask was allowed to come in contact with the organisms in the neck, by tilting the flask and then returning the contaminated fluid by tilting the flask back again, the broth became turbid after 24 hours. Pasteur made many other important contributions to microbiology and related sciences (Table 1-1) and for this reason has been called the Father of Microbiology.

Fig. 1-3. Louis Pasteur (1822–1895). (From *Microbiology*, Fourth Edition by Philip L. Carpenter. Copyright © 1977 by W. B. Saunders Company. Reprinted by permission of CBS College Publishing.)

GERM THEORY OF DISEASE

The theoretical explanations of infectious disease as proposed by Fracastorius in 1546 were not supported by experimental proof until 200 years later. Physicians, such as Semmelweis in Austria and Oliver Wendell Holmes in the United States, implored their physician colleagues to wash their hands before examining pregnant women. Both of these physicians had demonstrated that the agent of puerperal (childbed) fever was transmitted from an infected patient to an uninfected one by physicians who did not wash their hands. Their pleas were ignored by the majority of physicians who refused to believe they were unclean.

In 1857 Louis Pasteur formulated the theory of fermentation by microorganisms by demonstrating how microorganisms can convert sugar to lactic acid. From these experiments Pasteur theorized

Fig. 1-4. Swan-necked flasks were used by Pasteur to disprove spontaneous generation. Flask A, which is not swan-necked, contains sterile broth that is directly exposed to the air and becomes turbid after 16–24 hours. Microorganisms in the air settle into the broth where they reproduce and make the broth turbid. Flask B, which is swan-necked, also contains sterile broth. Because of its shape air, but not microorganisms, may enter the space above the broth. Microorganisms that are present in the air settle out by gravity in the curved neck of the flask. The broth in the swan-necked flask shows no turbidity even several days after exposure to air unless the flask is tilted and the broth makes contact with microorganisms.

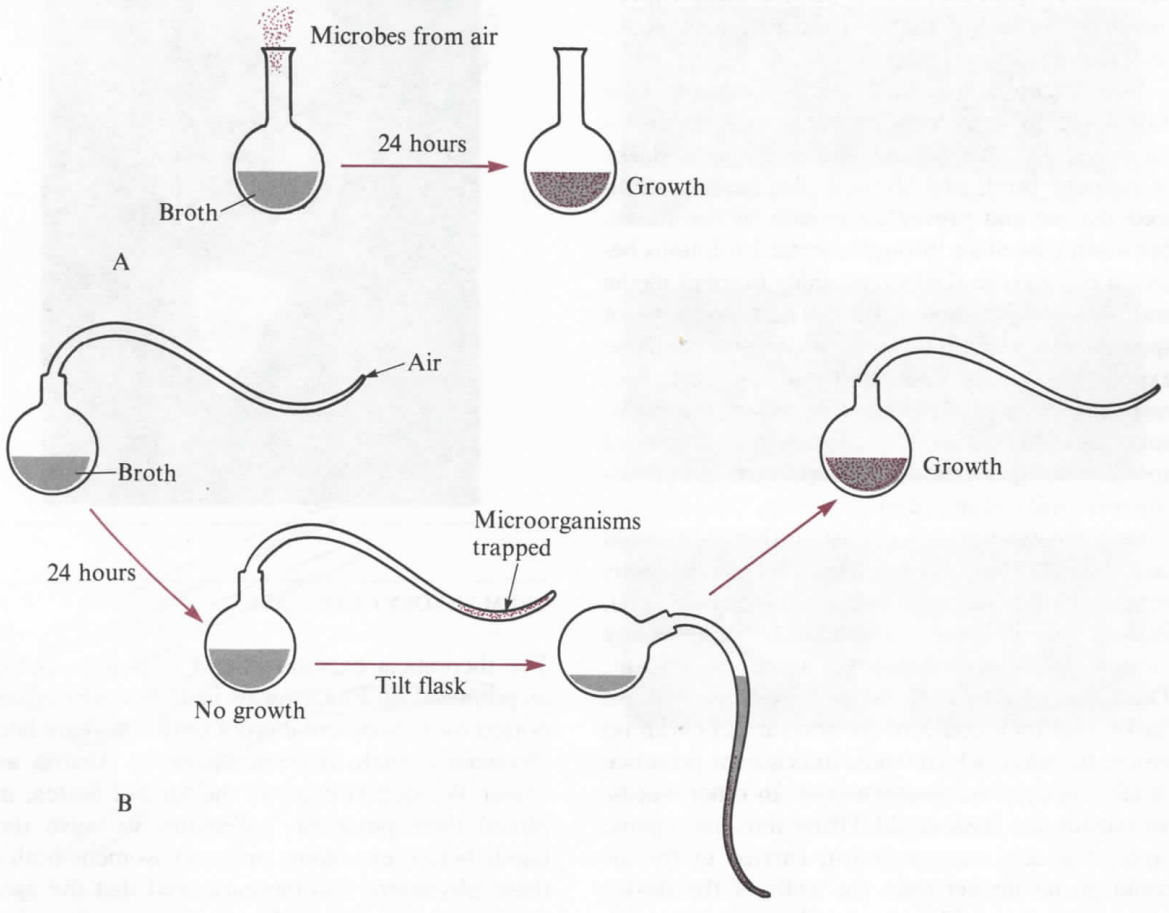


Table 1-1. Contributions of Louis Pasteur to Microbiology and Related Sciences

Development of attenuated vaccines for anthrax and chicken cholera
Immunization against rabies
Relationship of crystal structure to optical rotation
Study of diseases of wine and silkworms
Discovery of technique for selective destruction of microorganisms by heat (pasteurization)
Refutation of theory of spontaneous generation
Discovery of micoorganisms that live in the absence of air (anaerobes)
Contributions to understanding the causes of fermentation

that microorganisms could cause disease through similar chemical processes. In England at this time Joseph Lister, a surgeon, recognized the importance of Pasteur's experiments and proposed that infections of open wounds were due to microbes found in the air around the patient. It soon became Lister's policy to spray the air around the patient with phenol before surgical operations. Although this procedure dramatically decreased fatalities from surgical wound infections, Lister's results were not fully appreciated or even accepted by some fellow scientists.

In the late 1800s a German physician named Robert Koch (Fig. 1-5) demonstrated the relationship between microorganisms and infectious disease. Koch studied anthrax, a disease of cattle that can secondarily affect humans. He isolated the organisms from infected cattle in *pure culture* (the cultivation of a single species of microorganism) and then injected an aliquot of the pure culture into healthy animals, which subsequently became infected. The infectious agent was later isolated from these infected animals. This sequence of isolation, reinfection, and recovery of the infective agent is called *Koch's postulates*. By following Koch's postulates it was now possible to establish the causative agent of many infectious diseases. Although numerous scientists at that time were busily engaged in isolating and characterizing microorganisms, men like Koch and Pasteur were also interested in developing techniques that would be effective in destroying bacteria and thus reduce human misery.

Fig. 1-5. Robert Koch (1843–1910). (From K. L. Burdon and R. P. Williams, *Microbiology* [6th ed.]. New York: Macmillan, 1968. Copyright © 1968 by Macmillan Publishing Company.)

THE GOLDEN AGE OF MICROBIOLOGY (1870–1890)

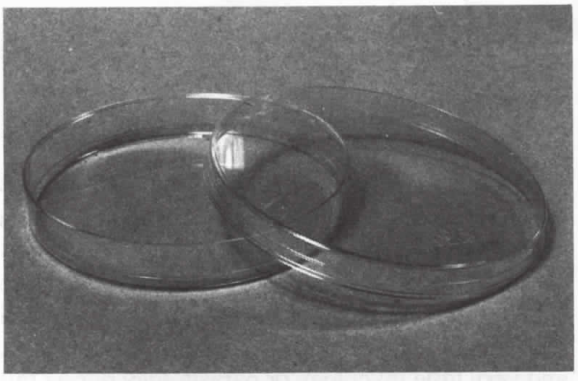
Isolation Techniques

It was in the Koch-Pasteur era (1870–1890) that tremendous strides were made in microbiology. It was during this era, called the *Golden Age of Microbiology*, that the microbial agents responsible for so many of the fatal diseases of that time were isolated (Table 1-2). There was no virology, immunology, and very little mycology or parasitology during this period. Perhaps one of the most important technical advances during this period was the discovery that *agar-agar* could be used with microbiological media for isolating microorganisms in pure culture. Agar-agar is a carbohydrate isolated from seaweed

Table 1-2. Disease-producing Bacteria Discovered During the Golden Age of Microbiology (1870–1890)

Year	Disease	Causative agent	Researcher(s)
1872	Anthrax	*Bacillus anthracis*	Rayer and Devaine, Pasteur and Koch
1873	Relapsing fever	*Borrelia recurrentis*	Obermeier
1874	Leprosy	*Mycobacterium leprae*	Hansen
1879	Gonorrhea	*Neisseria gonorrhoeae*	Neisser
1880	Pneumonia	*Diplococcus pneumoniae*	Pasteur, Sternberg
1880	Abscesses	*Staphylococcus aureus*	Pasteur, Ogston, Rosenbach
1882	Tuberculosis	*Mycobacterium tuberculosis*	Koch
1883	Cholera	*Vibrio cholerae*	Koch
1884	Diphtheria	*Corynebacterium diphtheriae*	Klebs, Loeffler
1884	Tetanus	*Clostridium tetani*	Nicolaier, Kitasato
1885	Food-borne illness, paratyphoid	*Salmonella choleraesuis* and related species	Salmon and Smith, Gärtner, Schottmüller
1887	Epidemic meningitis	*Neisseria meningitidis*	Weichselbaum
1887	Brucellosis	*Brucella melitensis* and other species	Bruce, Bang

Fig. 1-6. Petri dish. The bottom portion of the dish (*left*) can be partially filled with a solid media. The covering plate fits over the bottom portion. Petri dishes can be obtained presterilized and filled with sterilized media, or they may be unsterilized and filled with unsterilized media and then sterilized by autoclaving (steam under pressure).



that is stable to heat, is enzyme-stable, solid, transparent, and easily sterilized. Frau Hesse, the wife of a physician interested in the bacteriology of air, had been using agar-agar in her kitchen to prepare fruits and jellies. She suggested that agar-agar might be used in culture media. Dr. Hesse mentioned his wife's suggestion to Robert Koch, who adapted the new medium in his laboratory. The use of agar-agar in media made possible the isolation of single colonies on a solid medium, a technique that could not be accomplished with liquid media. This discovery, coupled with the design of the *Petri dish* (Fig. 1-6) in 1887 by J. R. Petri as a container for media, provided bacteriologists with a new tool for isolating bacteria in pure culture. Soon other microbiologists, using Koch's postulates and aided by these new isolation techniques, would make major contributions to an understanding of the microbial agents causing disease.

Staining

Visualization of microorganisms was enhanced by the discovery of various staining agents. Weigert, in 1878 was the first to stain bacteria using various aniline dyes. Further refinement in staining led to

Gram's stain (1884), which can be used for most bacterial species, and the Ziehl-Neelsen stain, which is used for staining the organism causing tuberculosis. Loeffler utilized methylene blue stain to identify the organism causing diphtheria.

Immunology

It had been recognized for more than 2000 years that individuals who recovered from some diseases could not "catch" the disease a second time. In 1796 Edward Jenner discovered that milkmaids infected with the mild variety of pox called *cowpox* were immune to the more severe form of the disease, *smallpox*. Jenner inoculated fluid from a cowpox pustule into a healthy boy and later infected the same boy with smallpox fluid. The boy did not contract smallpox.

In 1879 Pasteur, while studying cholera in chickens, noted that if chicken cholera bacteria were left on laboratory media for extended periods of time, they lost their virulence (they became attenuated). The attenuated bacteria, when injected into healthy chickens, not only failed to cause cholera but protected them from infection by fresh virulent strains. These experiments eventually led to our present-day vaccination techniques and methods of immunization. Pasteur later developed immunization procedures in the treatment of anthrax in animals and rabies in humans. Today all of us are aware of vaccination procedures used against such diseases as tetanus, diphtheria, polio, and whooping cough.

Elie Metchnikoff, working in Pasteur's laboratory, observed that certain white blood cells could ingest microorganisms or other small foreign matter. Metchnikoff firmly believed that immunity to infection was totally dependent on the special white blood cells (phagocytic cells) that digested other cells. Paul Ehrlich, an associate of Robert Koch, was also interested in resistance to infection. He believed that immunity to infection was due to certain soluble substances in the blood called antibodies. Both mechanisms have since been shown to be important in immunity and are referred to as the *cellular* and *humoral* mechanisms of defense, respectively.

VIROLOGY

Viral diseases such as smallpox, rabies, polio, and others had been known for centuries. The nature of the infectious agent involved, however, did not become apparent until the late 1890s. Iwanowsky took the fluids pressed from tobacco plants afflicted with tobacco mosaic disease and filtered them through porcelain filters to remove bacteria. The fluid still caused disease in new plants, and thus the infectious agent was recognized as a filterable material that did not contain bacteria. Loeffler and Frosch (1898) were the first to demonstrate a filterable agent as the cause of an animal disease called *foot-and-mouth disease*. They concluded that the filterable agent was an organism smaller than a bacterium and capable of replication. Twort (1915) discovered bacterial viruses from the observation of bacterial colonies growing on agar. Some of the colonies exhibited a glassy appearance. The material from the glassy colonies could be transferred to normal colonies and also cause the glassy appearance. d'Herelle in 1922 named the infectious material in these glassy colonies *bacteriophages* (bacteria eaters). d'Herelle also demonstrated that bacteriophages were capable of replication in their bacterial hosts.

Virology has seen many advances in the past 30 years, and most of the viral agents associated with infectious disease have been isolated and identified. In addition many vaccines have been successfully developed to prevent disease in mammalian hosts. Our current understanding of the molecular biology and genetics of viruses has led to important discoveries in mammalian genetics and the malignant processes caused by certain viruses.

CHEMOTHERAPY

In the late 1800s Paul Ehrlich, the German chemist, noted that dyes were selectively absorbed in some cell types and not in others. His observations led him to believe that certain chemicals taken into the body could selectively destroy bacteria and not affect normal body cells, a process he called "che-

motherapy." Ehrlich began a systematic search for a chemical that could be used against the microorganism that caused syphilis. After 606 trials an arsenical compound, which Ehrlich called *606*, was found to be effective in the treatment of syphilis. Until the discovery of penicillin, 606 (salvarsan) remained the major chemotherapeutic agent in the treatment of syphilis. Following Ehrlich's success many scientists began to test thousands of chemicals for antibacterial activity. In 1932, after years of research, Gerhard Domagk, a German chemist, prepared and tested a large number of dyes and discovered that the red dye Prontosil was highly effective in the treatment of numerous bacterial diseases. It was later discovered that in the body Prontosil is converted to a colorless derivative, called *sulfanilamide*, which was the active antibacterial component.

It had been known for several years before the turn of the century that certain bacteria and molds were capable of producing substances that killed or inhibited the growth of various types of microorganisms. In 1928 Sir Alexander Fleming discovered that a particular culture of *Staphylococcus* on an agar plate had become contaminated with mold from the air. Some of the bacterial colonies around one mold had stopped growing. The mold, later found to be *Penicillium notatum*, produced an antibacterial substance which Fleming called *penicillin*. In 1940 Chain and other scientists in Florey's laboratory in Oxford purified penicillin from culture fluids and demonstrated its potency. In 1942 penicillin was ready for injection into human subjects.

Selman Waksman, a Russian immigrant to the United States, during his undergraduate studies was interested in the types of microorganisms found in the soil. His work with a group of soil fungi called *actinomycetes* led to the discovery of the antibiotic actinomycin in 1940 and streptomycin in 1943. Streptomycin was soon shown to destroy bacteria not affected by penicillin, especially the tuberculosis bacillus. The experimentation and discoveries of Fleming and Waksman prompted a more extensive search for other antibiotics. After World War II a wider range of antibiotics, including tetracycline, chloramphenicol, and erythromycin, was discovered.

MODERN MICROBIOLOGY

Microbiology is now in the forefront of biological science. Many of the discoveries in microbiology have led to important discoveries in higher organisms and renewed interest and research in their biology. Much of this new interest has resulted from the development of gene-splicing techniques and the ability to produce large amounts of specific antibody (*monoclonal antibodies*). Gene splicing (recombinant DNA technology or genetic engineering) has already been used to produce various mammalian products in bacterial cells. Insulin, mammalian growth hormone, and interferon are just some of the mammalian proteins produced by bacterial cells. "Superbugs," or microorganisms containing foreign genes that code for highly degradative enzymes, have also been developed. These are being used to break down products such as petroleum compounds that are environmental pollutants and are resistant to microbial attack in soil or water.

Monoclonal antibodies have enabled scientists to pinpoint specific components that are part of the structure of all cells. For example, monoclonal antibodies can be used to find the viral or microbial component that is one of the determinants of infectious disease. This property will aid in the identification of viruses and will probably help us to develop better vaccines for the prevention of viral diseases. More detailed discussion of these aspects of modern microbiology will be presented in later chapters. Needless to say, the future of microbiology is very bright.

SUMMARY

Microbiology is a science that involves the study of microorganisms, that is, bacteria, fungi, certain al-

gae, and the protozoa, as well as the noncellular entities called viruses. Microbiology encompasses many branches of science including biochemistry, molecular biology, immunology, and others. The study of microorganisms has led to the development of various branches including food microbiology, industrial microbiology, environmental microbiology, and medical microbiology.

For centuries scientists believed that living things arose from nonliving material. This theory, called spontaneous generation, was finally refuted by Louis Pasteur in the late 1800s. The experiments developed by Pasteur and others before him in trying to refute spontaneous generation led to important discoveries concerning microbial life. The discovery that microorganisms can cause disease led to a period of unrivaled discoveries called the Golden Age of Microbiology. During this period many of the microbial agents causing disease were isolated and the development of vaccines was initiated. Soon after the Golden Age, developments in immunology, virology, and chemotherapy put medical microbiology in the forefront of science.

Modern microbiology, which has been recently enhanced by developments in biochemistry and genetics, is again in the forefront of science. The arms of microbiology are now touching and influencing most areas of biological science.

QUESTIONS FOR STUDY

1. List some of the achievements of Louis Pasteur.
2. Why is microbiology now in the forefront of the biological sciences?
3. Describe some of the experiments used to disprove spontaneous generation. Why were they refuted?
4. What contributions did Joseph Lister make to the field of microbiology?
5. What major advance or advances in the late 1800s do you think contributed to the isolation and identification of the agents of infectious disease?
6. What are Koch's postulates? Why are they important?
7. How were viruses first discovered to be smaller than bacteria?
8. Metchnikoff and Ehrlich proposed what theories to explain resistance to infection?
9. What is the significance of genetic engineering to medical science?
10. Who were the major early contributors to the development of chemotherapy?

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2. BIOCHEMISTRY OF MACROMOLECULES

OBJECTIVES

To describe the chemical composition of the macromolecules: proteins, polysaccharides, lipids, and nucleic acids

To explain what is meant by the primary, secondary, tertiary, and quaternary structure of a protein

To describe the types of bonds that influence the conformation of a protein

To explain the function of an enzyme and the factors affecting its activity

To describe the arrangement of lipids in the cytoplasmic membrane

To explain the function of the four basic types of nucleic acid molecules that are found in the cell

To describe the structure of the DNA molecule and the specific bonding that occurs between strands

To list the major monosaccharides, disaccharides, and polysaccharides that are found in biological systems

To define: isomer, peptide bond, dalton, denaturation, coenzyme, energy of activation, nucleotide, histone, glycosidic bond, purine, pyrimidine

Proteins
- Amino Acid Structure
- Peptide Bond Formation
- Protein Structure
  - Primary Structure
  - Secondary Structure
  - Tertiary Structure
  - Quaternary Structure
- Denaturation and Hydrolysis of Protein
- Enzymes
  - General Characteristics of Enzymes
  - Enzyme Activity
  - Factors Affecting Enzyme Activity
    - pH
    - Temperature
    - Substrate Concentration
    - Inhibitors
  - Classification of Enzymes
    - Based on Chemical Reaction
    - Based on Biological Activity
  - Applications of Microbial Enzymes

Lipids
- General Characteristics
- Simple Lipids
- Compound Lipids
- Lipids and the Cytoplasmic Membrane

Nucleic acids
- General Characteristics
- Structure of Deoxyribonucleic Acid
- Structure of Ribonucleic Acid
  - Messenger RNA
  - Transfer RNA
  - Ribosomal RNA
  - Viral RNA

Carbohydrates
- General Characteristics
- Monosaccharides
- Oligosaccharides
- Polysaccharides

All living things, including microorganisms, are composed of the four major macromolecules: proteins, nucleic acids, lipids, and carbohydrates. Each of these components has different structural and chemical properties that enable it to perform specific functions within the cell. It is our intention to discuss the chemical and physical properties of these macromolecules and to specify their overall role in the structure, genetics, and metabolism of the microbial cell. This discussion should help you understand the mechanisms used by microorganisms in the infectious disease process.

PROTEINS

Proteins are polymers (molecules consisting of a chain of repeating units, or monomers) of amino acids that vary in size, shape, and chemical properties. There are 20 naturally occurring amino acids (Fig. 2-1); their number and arrangement in the protein are responsible for the physical and chemical differences between different proteins. Proteins can play many different roles in the cell:

1. Catalysts. Many proteins in the cell are enzymes, which means that they act as catalysts or speed up chemical reactions by breaking and making chemical bonds. Enzymes will be discussed later in the chapter.

2. Structural support. Many components of the cell are made up of proteins that provide structural integrity and support. In microorganisms structural proteins can be found in the cell wall, in the cytoplasmic membrane, and in such organelles as mitochondria of more advanced microorganisms such as fungi and algae. They can also be found in the protein coat that surrounds the nucleic acid of viruses. Protein fibers called *microtubules* have been

found in the cytoplasm and adjacent to the cytoplasmic membrane of some organisms such as the protozoa. They are believed to lend structural support to the cytoplasmic membrane since a cell wall is absent in this group of microorganisms.

3. Antibodies. Antibodies are proteins produced by the immune system of vertebrates that help protect the host from disease-producing microorganisms and a variety of other harmful foreign substances.

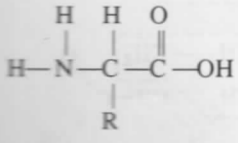
4. Contractile fibers. Contractile fibers have been found in microorganisms and are believed to be involved in the movement of some more advanced microorganisms as well as movement of the nuclear apparatus during cell division. Microtubules, for example, are components of flagella and cilia, which are involved in motility.

5. Toxins. Several microorganisms, especially bacteria, synthesize protein toxins that are injurious to mammalian tissue.

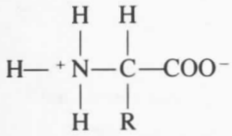
6. Transport. Proteins located in or near the cell membrane aid in the transport of a variety of organic or inorganic molecules into and out of the cell.

AMINO ACID STRUCTURE

The chemical properties of a protein are determined by the number, position, and chemical properties of its constituent amino acids. Amino acids are low molecular weight compounds that usually possess a free acidic or carboxyl (COOH) group and a free basic or amino (NH_2) group attached to a carbon atom called the *alpha carbon* (the carbon adjacent to the carboxyl carbon). They all have the general formula

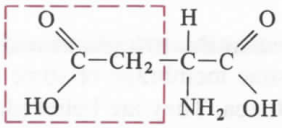
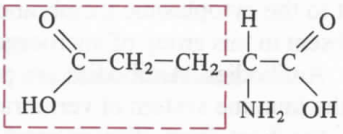
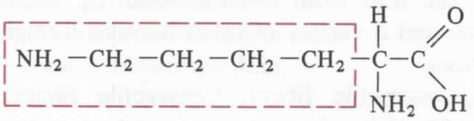
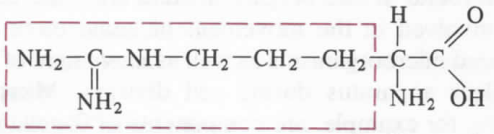
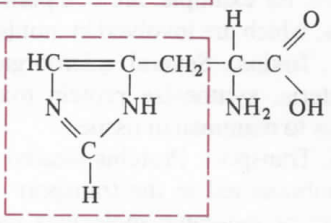
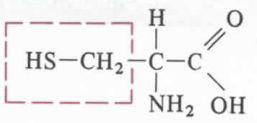
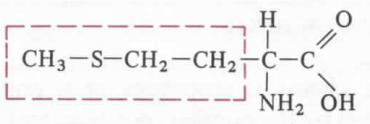
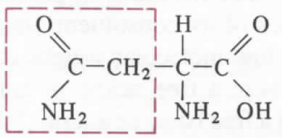
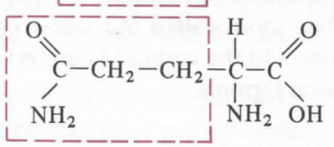
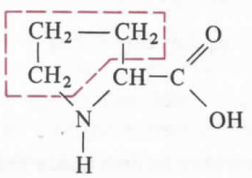


In water amino acids exist as dipolar ions containing a negatively charged (COO^-) group and a positively charged (NH_3^+) group or



The structure of the variable R group attached to the alpha carbon is responsible for the variation in chemical properties of the different amino acids. An amino acid containing only one amino group and one carboxyl group is neutral, but some amino acids contain R groups that possess acidic or basic properties. The structure of the various R groups is illustrated in Figure 2-1.

The amino acids are not flat molecules. The alpha carbon is situated in the center of the molecule, which is arranged in the shape of a tetrahedron (four-sided figure or pyramid) (Fig. 2-2). If one tries to rearrange the specific groups attached to the alpha carbon there are only two arrangements that are not superimposable (Fig. 2-2A). This is true only because the alpha carbon is asymmetrical. In other words the alpha carbon has four different chemical groups attached to it (except for glycine, which contains two hydrogens attached to the alpha carbon). The two arrangements that are not superimposable are called *isomers*. Structural isomers may be defined as molecules having the same molecular formula but different spatial arrangements of their atoms. By convention they are referred to as D-isomers and L-isomers. *D* and *L* refer to the position of the amino group: *D* (for *dextro-*, or *right*) to the right of the alpha carbon when the carboxyl group is written at the top; *L* (for *levo-*, or *left*) to the left of the alpha carbon. The conventional way of writing these isomers is illustrated in Figure 2-2B. The L-form is more frequently found in nature, but a few biological structures such as bacterial cell walls contain some D-amino acids.

Side chain (R group) characteristic	Amino acid	Chemical structure
Carboxylic (acidic)	Aspartic acid	
	Glutamic acid	
Amine bases (basic)	Lysine	
	Arginine	
	Histidine	
Sulfur containing	Cysteine	
	Methionine	
Amides	Asparagine	
	Glutamine	
Imino	Proline	

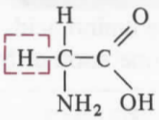
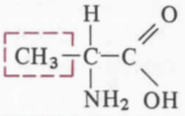
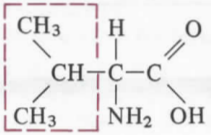
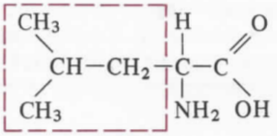
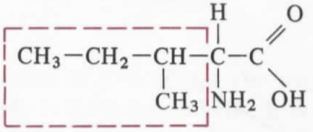
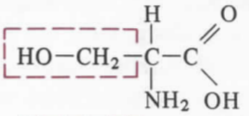
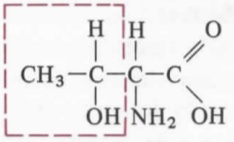
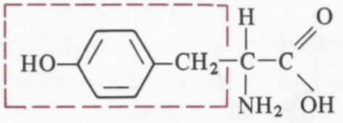
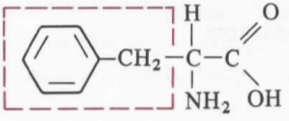
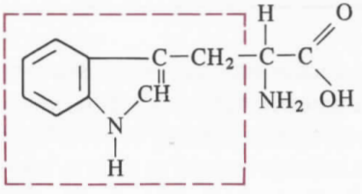
Side-Chain (R group) Characteristic	Amino acid	Chemical structure
Aliphatic, nonpolar	Glycine	
	Alanine	
	Valine	
	Leucine	
	Isoleucine	
Alcoholic, aliphatic, and aromatic	Serine	
	Threonine	
	Tyrosine	
	Phenylalanine	
	Tryptophan	

Fig. 2-1. Structure of the 20 naturally occurring amino acids. Area within dashed lines indicates R group. Note that dipolar nature of the amino acid is not indicated.

PEPTIDE BOND FORMATION

The amino acids in a protein are covalently linked to one another by a bond called the *peptide bond.* The peptide bond is formed through the interaction of the carboxyl group on one amino acid with the amino group of a second amino acid, with the subsequent release of one molecule of water:

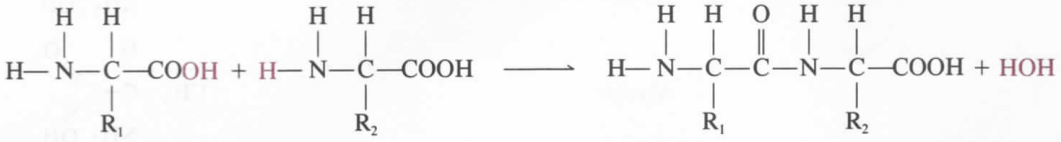
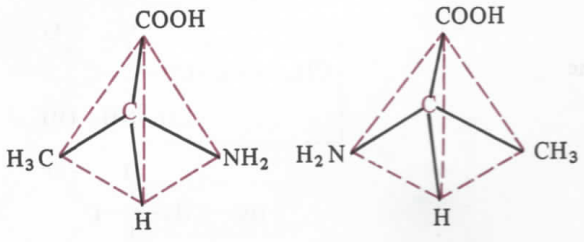


Fig. 2-2. Optical isomers of the amino acid alanine as they would appear in their tetrahedron shape. A. Spatial representation. B. Conventional representation. L-form has amino group on the left; D-form, on the right.



D-Alanine L-Alanine

A

D-Alanine L-Alanine

B

A chain of amino acids linked in such a manner is referred to as a *polypeptide*. If the chain is made up of 60 or more amino acids the polypeptide is usually referred to as a *protein*. Each of the amino acids in the protein are called amino acid *residues* because of the loss of one molecule of water during peptide bond formation. In biological systems not all peptides are polypeptides. The length of the peptide may vary from two amino acids (dipeptide) to 50 or more. Short peptides containing two to six amino acid residues are found in the cell wall of bacteria where they serve as linkage units between polysaccharide chains and thus provide structural stability to the cell wall. The cell wall component containing polysaccharides and short peptides is called the *peptidoglycan layer*.

PROTEIN STRUCTURE

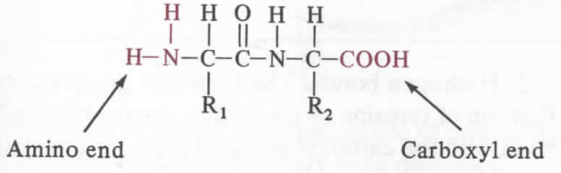
Proteins may be composed of one or more polypeptides and range in size from 6000 daltons to 6,000,000 daltons.* Many proteins, particularly structural ones, have nonprotein components that serve a variety of functions in the cell. Examples of these types of proteins in microorganisms are described in Table 2-1. The protein is not merely a linear chain of amino acids but can exist in different structural levels described as *primary, secondary, tertiary*, and *quaternary*.

*A dalton is an atomic mass unit (amu) and is defined as one-twelfth the mass of the most commonly occurring form of carbon, namely carbon 12 (^{12}C). Carbon has an atomic mass of 12.000 daltons; therefore, 1 dalton equals 1 amu.

Table 2-1. Proteins Classified According to Their Nonprotein Components

Class of protein	Nonprotein component	Example in microorganisms
Simple	None	Most enzymes
Glycoprotein	Carbohydrate	Part of the envelope surrounding the protein coat of some viruses; component of envelope surrounding cell walls of some bacteria
Nucleoprotein	Nucleic acids	Viral nucleic acid; chromosome of some advanced microorganisms
Lipoprotein	Lipids	Component of cytoplasmic membrane of microorganisms and cell wall of certain bacteria
Chromoprotein	Colored group such as heme or riboflavin	Cytochromes, which are involved in oxidation-reduction reactions; enzymes such as catalase and peroxidase

Fig. 2-3. The polypeptide. The N-terminal has a free amino (NH_2) group and is usually represented as being to the left, while the free carboxyl group (C-terminal) is located on the right.



Primary Structure

The fixed sequence of amino acids in a polypeptide is referred to as the primary structure. It is the sequence of amino acids that not only distinguishes one polypeptide from another but subsequently determines the conformation of the protein molecule. Every polypeptide has two terminals: the N-terminal or free amino end and the C-terminal or free carboxyl end (Fig. 2-3).

The sequence of amino acids is known for several large proteins such as hemoglobin and insulin. This sequence is directly related to the sequence of bases that appears in the genetic material of the cell and is described in Chapter 5. Analysis of some of the protein from microorganisms has indicated that the protein may be grouped into evolutionary families and thus play a role in the classification of microorganisms.

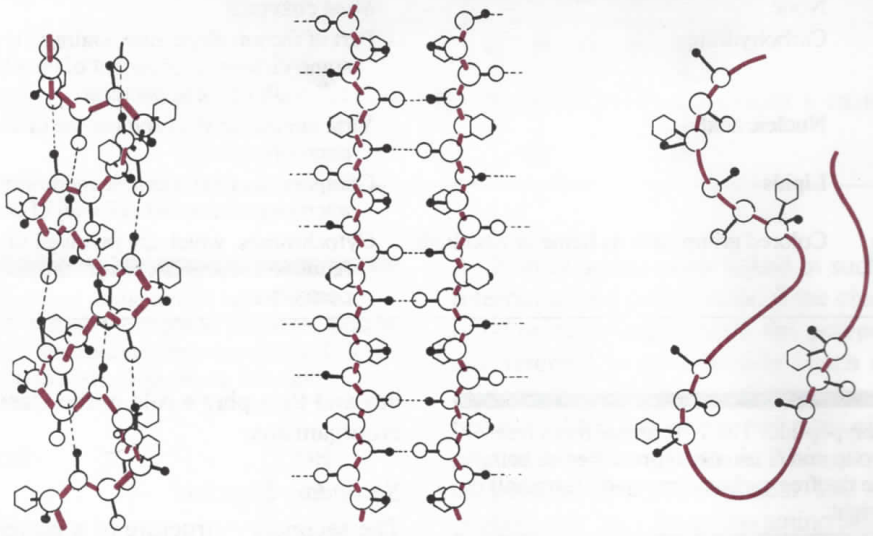
Secondary Structure

The secondary structure of a protein is the way in which the polypeptide is folded and bent. It is the nature of macromolecules in solution to assume a configuration that requires the least amount of energy while maintaining structural stability. Proteins may assume three different conformations depending on their amino acid composition: the random coil, the pleated sheet, and the alpha helix (Fig. 2-4). The alpha helix, the most commonly encountered conformation, resembles a spiral chain in which each turn of the helix contains 3.7 amino acid residues. The stability of the helix is maintained by hydrogen bonds involving the C═O of one amino acid residue and the N—H of an amino acid residue three amino acids down the chain. As you can see from Figure 2-4 the R groups extend away from the helix and are not in its center. This has important consequences for the tertiary structure of the polypeptide.

Tertiary Structure

The helical polypeptide in an aqueous solution is not sufficiently stabilized by hydrogen bonding and requires folding and bending. Bends in the polypeptide appear wherever proline or hydroxyproline appear in the molecule. The bending is due to the fact

Fig. 2-4. Three conformational forms of proteins. (● = hydrogen; ⬡ = R group; ○ = C═O.)



that when a peptide bond is formed involving proline or hydroxyproline no hydrogen bond is available for bonding to a C═O group three amino acids down the chain. Bends destroy the alpha helical configuration of the polypeptide, and a protein with many prolines cannot form a normal helical structure. The folding of the protein is helped by the bends that appear in the molecule but is more affected by the characteristics of the R groups. Interactions between specific R groups can result in the following covalent and noncovalent bonds, which have the greatest influence on the configuration of the protein (Fig. 2-5):

1. Disulfide (S—S) bonds. Disulfide linkages are covalent bonds resulting from the interaction between sulfhydryl (SH) groups on cysteine residues. The disulfide may appear within a single chain or between two chains. Disulfide bonds are major contributors to chain folding and stabilization of the three-dimensional shape of the protein.

2. Hydrogen bonds. The hydroxyl group on the R group of tyrosine or serine may form a hydrogen bond with the carboxyl group of glutamic or aspartic acid.

3. Salt bonds. Salt bonds are electrostatic interactions between amino acids with ionizable groups such as NH_3^+ of glutamine and the carboxyl (COO^-) group of aspartic or glutamic acid.

4. Hydrophobic bonds. Hydrophobic bonds are interactions between chemical groups that "dislike" water. The aromatic R groups of phenylalanine and tryptophan are attracted to each other because of their repulsion of water. The hydrophobic groups of a polypeptide have a tendency to seek each other out and produce a folded molecule in which the hydrophobic groups remain on the inside of the protein while the hydrophilic ("water-loving") groups, such as aspartic acid and glutamic acid, remain on the surface of the protein and in contact with water.

Fig. 2-5. Structure of a protein showing the types of interactions that contribute to the tertiary structure. A. Salt bond. B. Hydrophobic bond. C. Disulfide bond. D. Hydrogen bond.

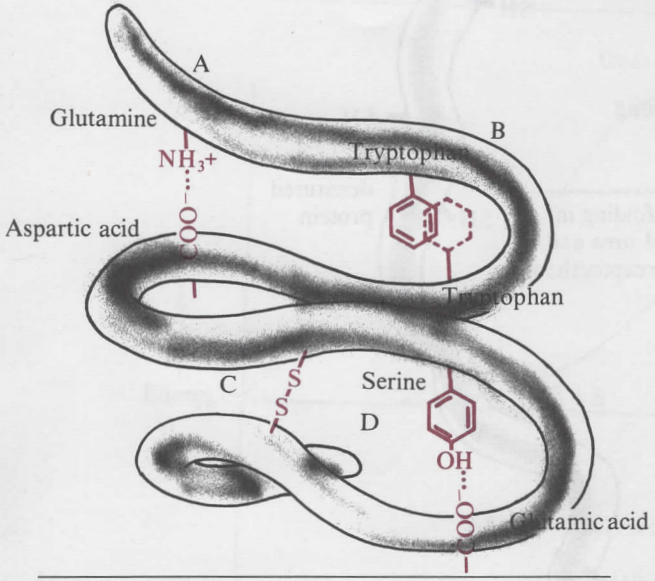
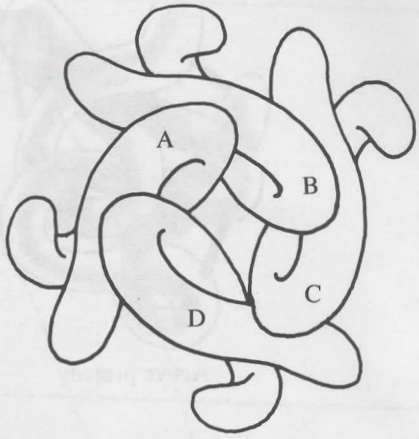


Fig. 2-6. Quaternary structure of protein. This globular protein consists of four polypeptides (*A*, *B*, *C*, and *D*) that interact with each other to create the quaternary structure. Quaternary structure may involve more than four polypeptides, depending on the protein.



5. Proline residues. The importance of proline in bending the protein has already been discussed.

Quaternary Structure

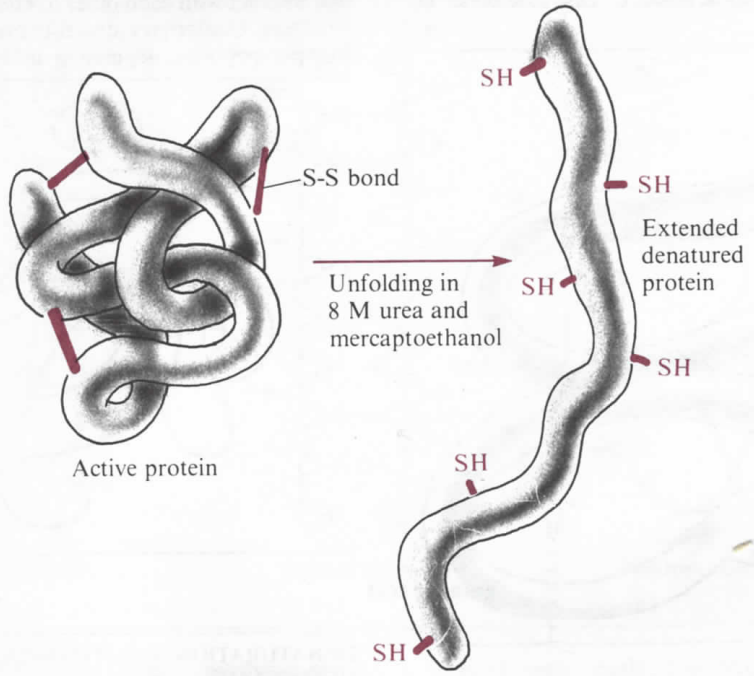
Many proteins are made up of two or more polypeptides. The spatial arrangement and interaction of these polypeptides is referred to as the quarternary structure of the protein. A folded polypeptide still has R groups projecting from its surface and in contact with water. Hydrophobic interactions between the R groups of two polypeptides can bring them together and help reduce their interaction with water (Fig. 2-6). A similar relationship can be observed when oil is allowed to stand in water for a period of time. The oil forms droplets, which later coalesce to form larger droplets and thus shelter the hydrophobic R groups from water.

DENATURATION AND HYDROLYSIS OF PROTEINS

Protein denaturation is a process in which the macromolecule, because of some chemical or physical action, has lost its biological activity but no peptide bond has been broken. Denaturation involves the breaking of bonds involved in maintaining the conformation or three-dimensional structure of the protein. Many proteins are held together by S—S linkages. The addition of a reducing agent such as mercaptoethanol can break the S—S bond and promote the formation of sulfhydryl (SH) groups, which alters the conformation of the protein (Fig. 2-7). Altering the pH can affect salt linkages, hydrogen bonds, and hydrophobic bonds and thereby promote denaturation. Denaturation of a protein is often reversible if the protein is a single polypeptide but not if the protein has multiple subunits.

The chemical breakdown of a molecule by the addition of water is called *hydrolysis*. During peptide bond formation water is released, but to split

Fig. 2-7. Effect of chemical reducing agents on the structure of protein containing sulfur–sulfur bonds.



the peptide bond water must be added across it. Hydrolysis is a common chemical process in living cells because it permits large molecules to be broken into smaller units that can be used in a variety of different processes by the cell.

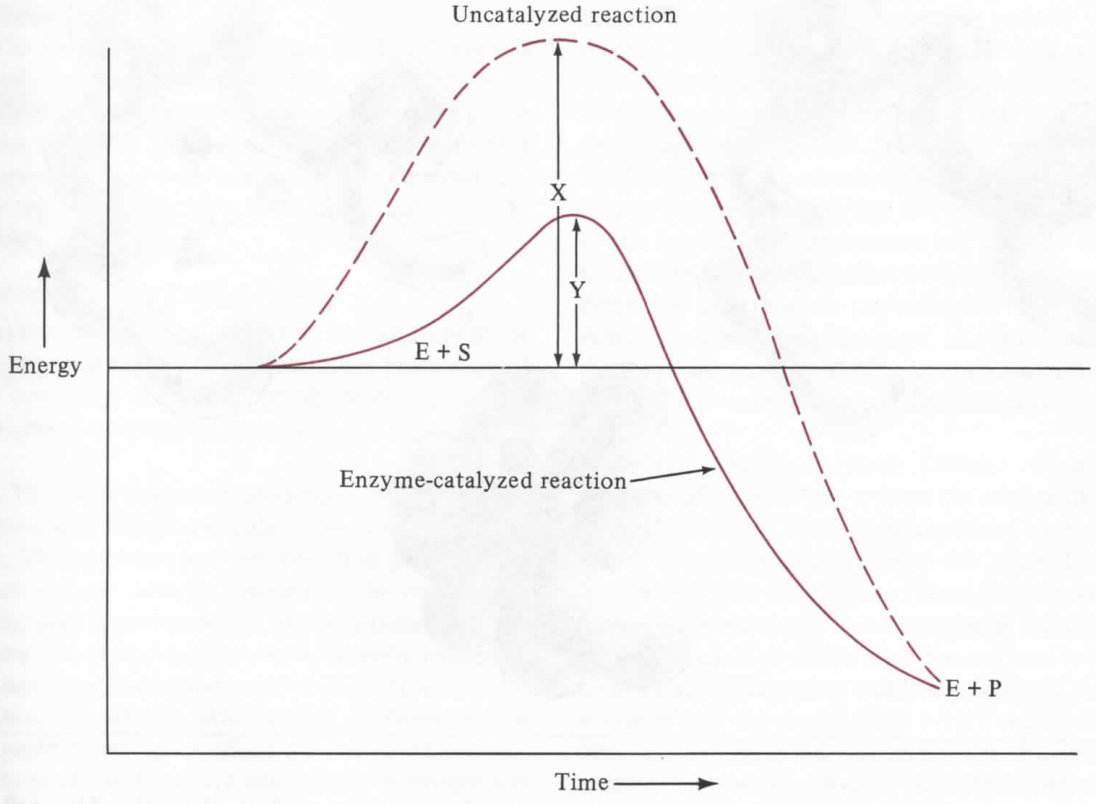
ENZYMES

The speed at which living cells grow, divide, move, and carry out various metabolic reactions is a direct result of the activity of a special group of proteins called *enzymes*. In addition to their obvious importance to the cell, enzymes are of special interest to research and clinical microbiologists. The research microbiologist measures various enzymes in the microbial cell to elucidate metabolic pathways or other cellular activities. A researcher may also use enzymes to synthesize products outside the cell (in vitro) that would ordinarily be synthesized in the cell (in vivo). In addition, some researchers determine the physical and chemical properties of enzymes as well as other proteins to learn more about the mechanisms by which proteins carry out their various metabolic functions. The clinical microbiologist may examine the specific enzymatic activity of microorganisms as a means of microbial identification and may also measure changes in host enzymes induced by disease-producing microorganisms.

General Characteristics of Enzymes

All enzymes are proteins and are globular in shape. They range in size from the smaller polypeptides having molecular weights of 6000 to 12,000 daltons to the large multiple subunit enzymes having molecular weights of 2,000,000 daltons or more. Many enzymes are not active unless other organic mole-

Fig. 2-8. Energetics of a chemical reaction. The substrate (*S*) is at a particular energy level but cannot be converted to product (*P*), which has a lower energy level, until energy (activation energy) is put into the system. In an uncatalyzed reaction the activation energy (*X*) is considerably larger than the activation energy (*Y*) for an enzyme-catalyzed reaction.



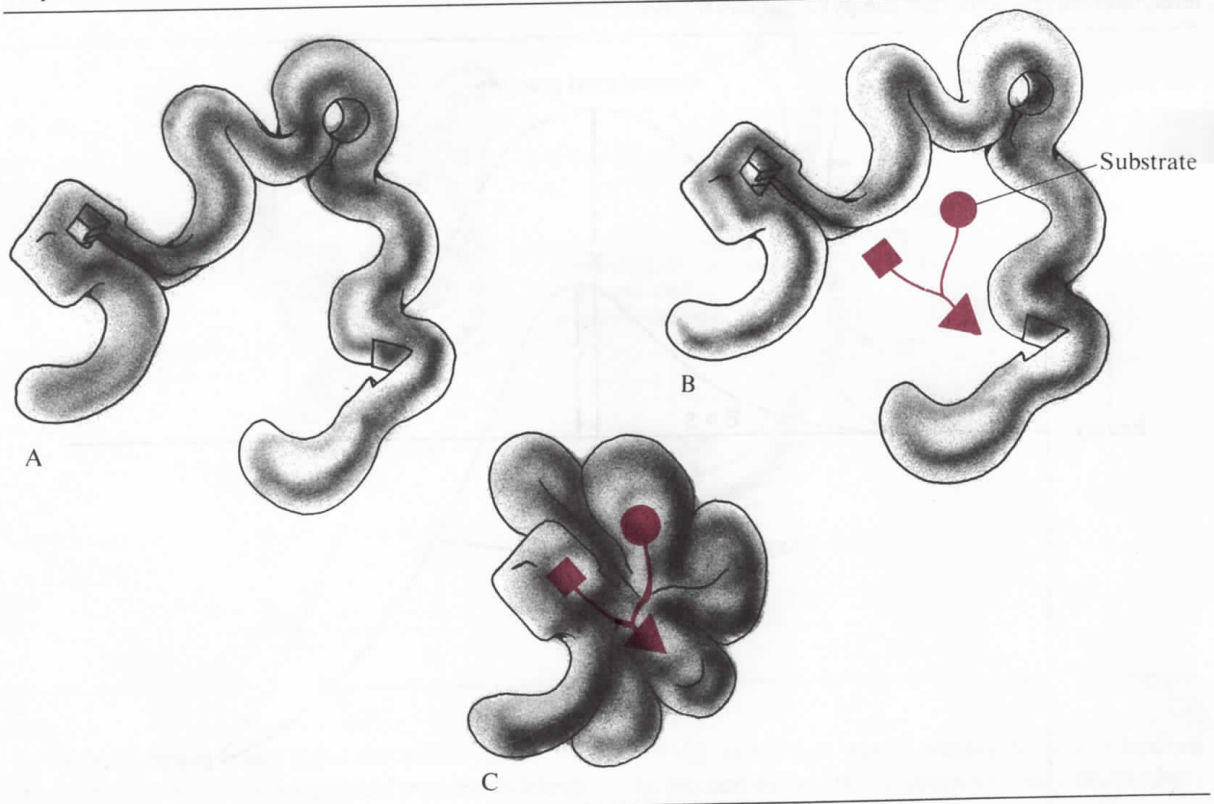
cules or inorganic ions called *cofactors* are also present on the protein. The protein portion of the enzyme is called the *apoenzyme*, while nonprotein organic cofactors attached to the protein are called *coenzymes* and are invariably vitamins such as thiamine, riboflavin, biotin, niacin, and others. Inorganic cofactors are metal ions such as calcium, zinc, manganese, magnesium, molybdenum, and iron. Coenzymes play very important roles in the enzymatic process such as the carriage of protons and removal of carboxyl groups.

Enzyme Activity

Many enzymes show a high degree of specificity in the type of chemical reactions they catalyze. This specificity may be for one of the reactants, such as the sugar glucose, or for specific functional groups on the reactants, such as amino or phosphate groups, or for optical isomers of the reactants, such as the L-form or D-form of an amino acid.

In every chemical reaction there are reactants and products. The reactants in enzyme-catalyzed reactions are commonly referred to as *substrates*.

Fig. 2-9. Active site on the enzyme molecule. Substrate has chemical units that have an affinity for sites on the enzyme and alter its conformation. The substrate-enzyme complex has a lower activation energy than the substrate alone.



In nearly all uncatalyzed reactions the conversion of a substrate to a product is a very slow process. The most notable exception is hydrogen peroxide (H_2O_2), which breaks down into oxygen plus water in a relatively short time without the aid of an enzyme catalyst:

$$2H_2O_2 \longrightarrow 2H_2O + O_2$$

The reason for the slow conversion of most substrates (S) to products (P) is that bond making and bond breaking require high levels of energy referred to as *activation energy* (Fig. 2-8). Activation energy can be supplied in the form of heat, but the heat required to make or break many chemical bonds is detrimental to the cell. The cell has through evolutionary steps overcome this barrier through the development of enzymes. Enzymes lower the amount of energy required to make or break bonds because they possess sites where reactants can interact or a single reactant can be altered. A typical enzymatically catalyzed reaction can therefore be represented as

$$E + S \rightleftharpoons ES \rightharpoonup P + E.$$

The substrate (S) interacts with the enzyme (E) to form an enzyme-substrate complex (ES), which has a lower activating energy than the substrate alone (Fig. 2-8). The site where the substrate binds

on the enzyme is called the *active site*. It is at the active site that bond breaking and bond making take place because of the interaction of amino acids and cofactors with the substrate (Fig. 2-9). As the product is formed it is released by the enzyme and the enzyme is able to engage another substrate molecule.

The specificity of the enzyme for its substrate is based on the shape of the protein, which in turn influences the arrangement of the amino acids at the active site. It is this amino acid arrangement that determines what substrate bond is to be modified. This type of specificity is often compared to a lock-and-key relationship.

Factors Affecting Enzyme Activity

Enzymes are proteins subject to the same chemical and physical factors that affect other proteins, and any condition that alters the shape of the protein also affects its catalytic activity.

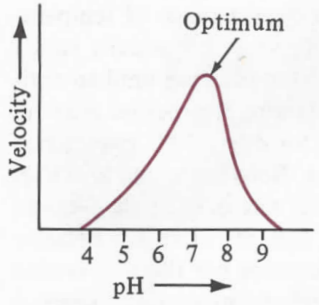
pH. The most favorable condition under which an enzyme can function is called the *optimum* condition. The hydrogen ion concentration greatly influences enzyme activity because of the many ionic amino acid R groups found on the protein. The optimum activity of the enzyme is directly related to the nature of the amino acids at the active site. Alterations in pH can influence the conformation of the protein and hence affect the rate at which substrate binds to the active site. As the hydrogen ion concentration is increased, COO^- groups are protonated (H^+), S—S bonds are converted to sulfhydryl (SH) groups, and NH_2 groups are protonated to form NH_3^+. In very acid solutions the protein is therefore positively charged, while at alkaline pH's the protons are removed from the protein, which becomes negatively charged. The pH range for those enzymes involved in metabolic reactions in microbial cells is usually between 5.0 and 8.0 (Fig. 2-10A).

TEMPERATURE. The optimum temperature for enzymes found in mammalian systems is 37°C, and this temperature also applies to most of the enzymes found in microorganisms that infect humans. Microorganisms found in hot springs possess enzymes that are active above 40°C, while microorganisms indigenous to cold climates have enzymes that are active at 0°C; however, these groups do not infect humans. When the activity of an enzyme is measured over a range of temperatures (Fig. 2-10B) it can be seen that activity varies directly with increases in temperature until an optimum range is reached. During this period enzyme activity usually doubles for every 10°C rise; hence the straight-line function. Between y and z in Figure 2-10B there is a slower rate of increase because two forces are at work. The rate increases because of the increase in temperature but the rate is also slowed because at the higher temperature there is a degree of denaturation (unfolding of the protein). After the optimum temperature has been reached, the enzyme activity decreases precipitously because of the increased rate of denaturation.

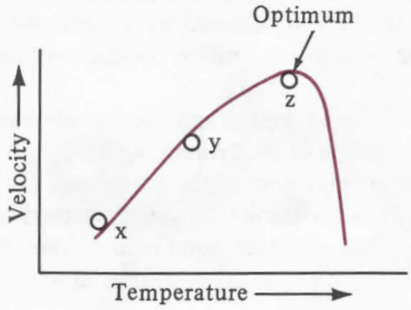
SUBSTRATE CONCENTRATION. When substantial amounts of enzyme are present the rate of the catalyzed reaction is directly proportional to the substrate concentration, provided all other factors, such as pH and temperature, have been standardized. As the substrate concentration is increased a point is reached at which all of the enzyme is in the form of an ES complex and the velocity of the reaction is at a maximum (Fig. 2-10C) and independent of the substrate concentration. Further increases in substrate produce no further increase in activity, and only by adding more enzyme can the maximum velocity be increased.

INHIBITORS. In medicine the control of some infectious diseases is generally based on the control of microorganisms. Agents that affect enzyme activity also affect the growth and metabolism of microorganisms. Science is continually looking for chemical agents that selectively inhibit microbial enzymes without affecting the enzymes of the host. Most of the chemical agents that show this selective inhibition are antibiotics, which are discussed in Chapter 11. Inhibitors of enzyme activity are of two types: competitive inhibitors and noncompetitive inhibitors. Competitive inhibitors compete

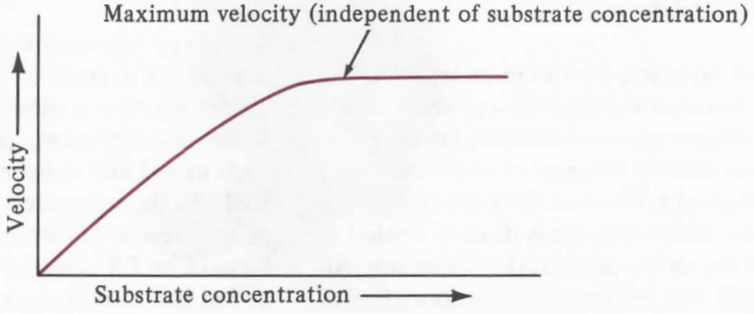
Fig. 2-10. Influence of various factors on enzyme activity. A. pH. B. Temperature. C. Substrate concentration. D. Inhibitors.



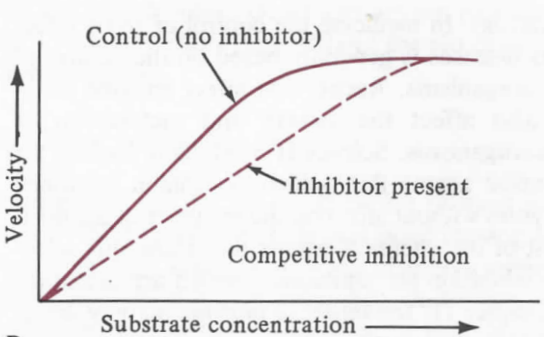
A



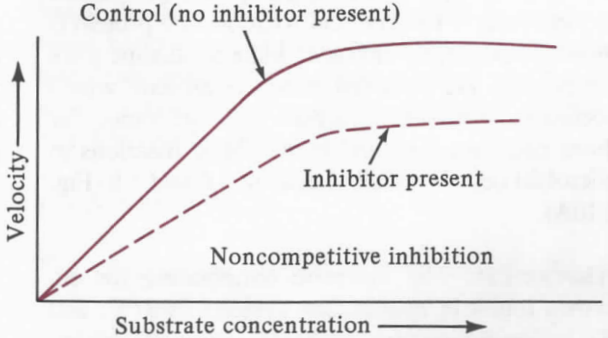
B



C



D



with the normal substrate for the active site on the enzyme molecule. The competitive inhibitor has a structure similar to the natural substrate, which enables it to bind to the enzyme but is different enough that it is not converted to the normal product. The inhibitor forms a dissociable enzyme-substrate complex just like the normal substrate. In competitive inhibition the velocity of the reaction is slowed, but the maximum velocity that is characteristic of the reaction in the absence of inhibitors can be obtained if the substrate concentration is increased (Fig. 2-10D). Noncompetitive inhibitors do not bind to the active site but at another site. The noncompetitive inhibitor affects the binding of substrate to the active site, probably by inducing some conformational change in the enzyme. Even if the substrate binds to the active site, in the presence of inhibitor a product may be formed very slowly or not at all. Increasing the substrate concentration will have no effect on enzyme activity in the presence of noncompetitive inhibitors as it would for competitive inhibitors (Fig. 2-10D).

Classification of Enzymes

BASED ON CHEMICAL REACTION. Except for enzymes such as pepsin and chymotrypsin that were identified and named many years ago, enzymes end in the suffix -ase: protease, lipase. The first part of the name usually indicates the type of substrate, chemical group, or type of chemical reaction that is catalyzed by the enzyme. Current nomenclature divides all enzyme-catalyzed reactions into the following groups:

1. Oxidoreductases are involved in electron (hydrogen) transfer reactions.
2. Transferases transfer specific groups such as aldehydes or phosphates from one substrate to another.
3. Hydrolases add water across chemical bonds to be cleaved or hydrolyzed.
4. Lyases remove chemical groups from substrates forming double bonds or add chemical groups to double bonds.
5. Isomerases rearrange certain compounds to form their structural isomers.
6. Ligases produce bonds accompanied by the cleavage of adenosine triphosphate (ATP).

Note that some of the more common names for enzymes are still used and are appropriately descriptive. Enzymes called lipases, for example, act on lipids, but current enzyme nomenclature would describe them as glycerol ester hydrolases.

BASED ON BIOLOGICAL ACTIVITY. Some enzymes synthesized by the cell remain within the cell to carry out specific reactions and are called *endoenzymes*, while others are released from the cell into the surrounding environment and are called *exoenzymes*. Many microbial cells release exoenzymes that attack macromolecules and convert them into smaller units that can be selectively absorbed by the cell. Starch and cellulose, for example, are high molecular weight macromolecules made up of glucose residues and are characteristically found in plants. Some microbial cells can release enzymes that degrade these polymers into individual glucose units that are small enough to pass through the microbial cytoplasmic membrane and provide a source of energy for the cell.

The prefixes *endo-* and *exo-* have also been used to describe the site of enzyme activity on macromolecules. Exoenzymes, for example, catalyze reactions involving terminal residues on the macromolecule, while endoenzymes catalyze reactions involving residues found in the interior of the molecule.

Applications of Microbial Enzymes

Enzymes produced by microorganisms have a variety of uses, and some are commercially prepared on a large scale. Protease, an enzyme used to degrade protein by splitting peptide bonds, is obtained from the bacterium *Bacillus licheniformis*. Protease is used as a cleaning aid in detergents. Proteases obtained from other bacteria and from

fungi can also be used as digestive aids in animal feeds and as meat tenderizers. Alpha-amylase, glucamylase, and glucose isomerase are bacterial enzymes used to convert starch into a sweetener (high-fructose corn syrup) that is rapidly replacing sucrose in soft drinks.

Proteolytic enzymes, such as streptokinase, are produced extracellularly by species of the bacterium *Streptococcus* and have been used clinically to dissolve fibrin clots because of their ability to convert blood plasminogen to active plasmin, the proteolytic component that breaks down fibrin molecules. Streptokinase has recently been approved for use in the treatment of deep venous thrombosis and massive pulmonary embolisms, conditions that ordinarily require the surgical removal of vascular clots.

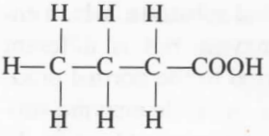
LIPIDS

GENERAL CHARACTERISTICS

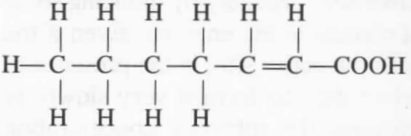
Lipids are heterogeneous substances that are insoluble in water but soluble in organic solvents such as chloroform, benzene, and alcohol. Lipids perform several functions in the cell. They are used as (1) a source of energy when carbohydrates are unavailable, (2) a structural component of the cell wall of some bacteria, (3) structural components of the cytoplasmic membrane, and (4) the protective structure that surrounds some viruses. In microbial systems most of the lipids are actual or potential esters of fatty acids that upon hydrolysis release fatty acids and alcohol. Fatty acids have the general formula

$$CH_3(CH_2)_nCOOH$$

The value of n in naturally occurring lipids ranges from 10 to 20. Fatty acids may be saturated or unsaturated, branched, or cyclic, but most in microbial species are either saturated or unsaturated. A saturated fatty acid is one in which all of the hydrocarbons are methylene (CH_2) units, as in the following fatty acid, butyric acid:



Some of the more commonly encountered saturated fatty acids are lauric acid (12 carbons), palmitic acid (16 carbons), and stearic acid (18 carbons). Fatty acids in which there are one or more double bonds, such as the following, are referred to as unsaturated:



Some of the more commonly encountered unsaturated fatty acids are oleic acid (18 carbons), linoleic acid (18 carbons), and arachidonic acid (20 carbons).

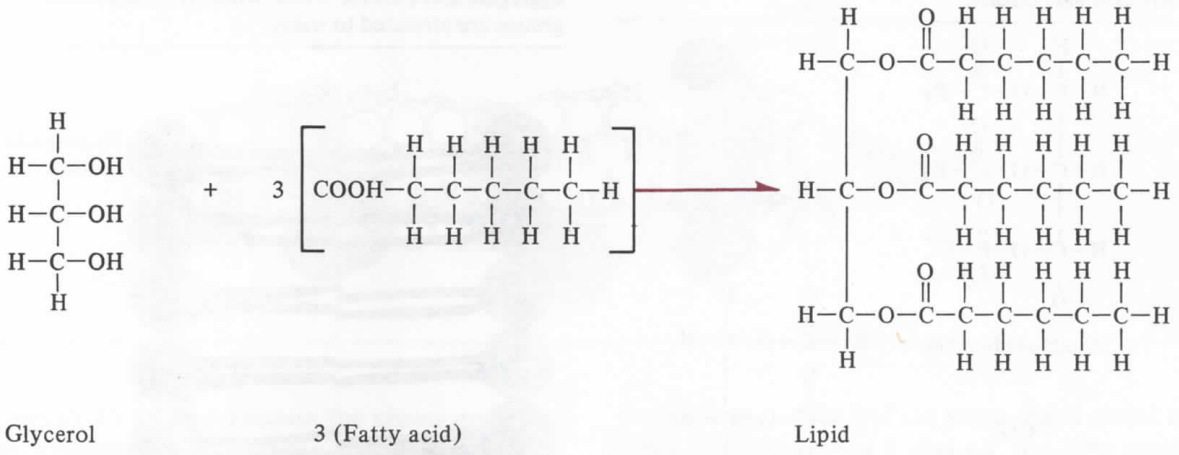
SIMPLE LIPIDS

Most fatty acids do not appear free in the cell but are covalently bound to other groups. Many of the simple lipids are esters resulting from the interaction of fatty acids with various alcohols, especially glycerol. Glycerol contains three hydroxyl groups, each of which can be covalently attached to a fatty acid. The linkage in an ester bond is between the hydroxyl group of the alcohol and the carboxyl group of the fatty acid (Fig. 2-11). Free fatty acids are a source of energy in the cell when other energy sources have been exhausted.

COMPOUND LIPIDS

Those lipids that contain phosphorous, nitrogen, or sulfur as additional elements in their structure are referred to as *compound lipids*. Compound lipids function as structural units within the cell and are

Fig. 2-11. Chemical reaction involved in the formation of a lipid.



characteristic of membranes. A compound lipid called *phosphatidic acid* (Fig. 2-12) is a phosphoglyceride and is a source of several types of compound lipids such as phosphatidylcholine, phosphatidylethanolamine, and phosphatidylserine. The presence of phosphate groups endows the lipid with a water soluble (hydrophilic) end, which in addition to the hydrophobic (hydrocarbon chains) portion of the molecule makes it double headed in respect to its water solubility. Some compound lipids are associated with macromolecules such as proteins (lipoproteins).

LIPIDS AND THE CYTOPLASMIC MEMBRANE

When phosphoglycerides are placed in a water medium the hydrophobic ends tend to aggregate to avoid water while the hydrophilic groups tend to associate with water (Fig. 2-13). The two-headed nature of lipids is important in the construction of cell membranes, and one arrangement that is associated with this characteristic is the phospholipid bilayer. The phospholipid cell membrane is also composed of other molecules, the most important of which is protein. Lipids make up approximately 40 percent by weight of the cytoplasmic membrane; protein, 60 percent. According to available evidence the proteins are believed to be embedded in the phospholipid bilayer as shown in Figure 2-14.

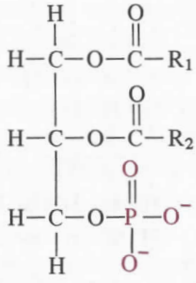
The membrane surrounding the cytoplasmic contents is referred to as the *plasma membrane* (cytoplasmic membrane) and because of its universal nature is called a *unit membrane*. In eukaryotic microorganisms, such as fungi and protozoa, there are more complex membranes including those surrounding the nucleus, the mitochondria, and other cellular organelles. The membrane system of all organisms is a barrier to the transport of certain molecules. This function is described in more detail in Chapter 4.

NUCLEIC ACIDS

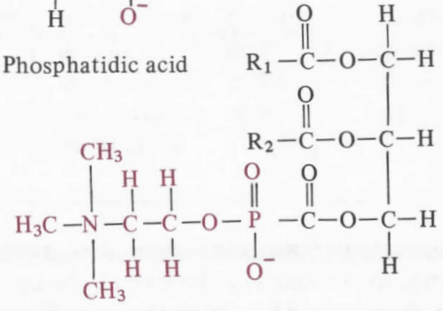
GENERAL CHARACTERISTICS

Two types of nucleic acids are found in living cells: deoxyribonucleic acid (DNA) and ribonucleic acid (RNA). In viruses one or the other but not both is present. DNA is the information-carrying molecule in the microbial cell, but this function can also be handled by RNA, which is the hereditary material of a few viruses.

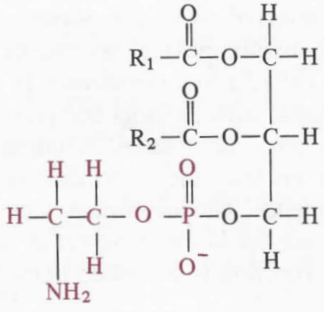
Fig. 2-12. Structure of the compound lipid phosphatidic acid and some of its derivatives. R_1 and R_2 represent fatty acid side chains.



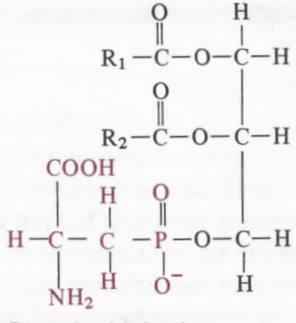
Phosphatidic acid



Phosphatidylcholine

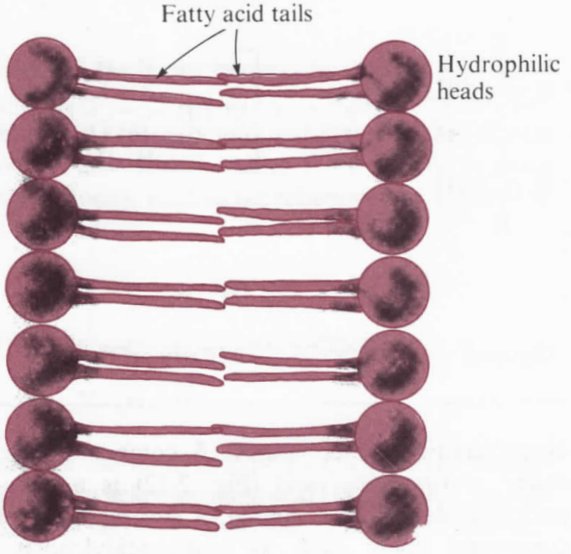


Phosphatidylethanolamine



Phosphatidylserine

Fig. 2-13. Aggregation properties of a lipid in an aqueous medium. The hydrophobic fatty acids tend to aggregate and exclude water, while the hydrophilic groups are attracted to water.

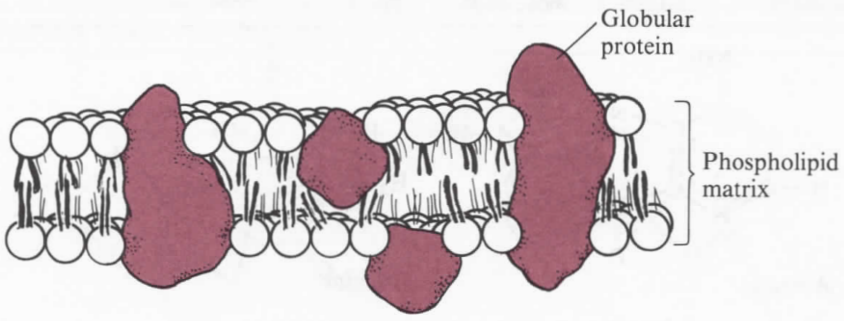


The nucleic acids are long chains of monomeric repeating units called *nucleotides*. A nucleotide is made up of a nitrogenous base, either a purine or pyrimidine (Fig. 2-15), plus a sugar phosphate. The nucleic acid is, therefore, a polymer made up of nucleotide repeating units and has a molecular weight ranging from 1×10^4 to 1×10^9 daltons.

STRUCTURE OF DEOXYRIBONUCLEIC ACID

The DNA molecule consists of two linear chains of nucleotides (some viruses contain a single-stranded DNA). Each strand can be viewed as consisting of nucleotides connected to each other by a phosphate ester linkage (Fig. 2-16). The phosphate links the 5′-hydroxyl of one nucleotide to the 3′-hydroxyl of the adjacent nucleotide. The ends of the nucleotide chains are different in that one end has a free 3′-hydroxyl while the opposite end has a free 5′-hydroxyl. The second DNA chain is comple-

Fig. 2-14. Structure of the cytoplasmic membrane. Globular proteins are embedded in a phospholipid matrix.



mentary to the first because the chains are linked by hydrogen bonds between specific bases. Guanine, for example, pairs with cytosine and adenine with thymine. The ratio of guanine to cytosine and adenine to thymine in the double-stranded DNA molecule is always 1.0 because of this specificity. The percentage of each base pair, however, varies from one species to another, and this variation has been used as one means of classifying microorganisms (see Genetic Analysis in Chapter 4). A single hydrogen bond is a weak bond, but if you consider that a DNA molecule may possess 1 to 4×10^7 nucleotides, the number of hydrogen bonds present is sufficient to keep the strands together. The energy required to split an individual hydrogen bond is quite small, and the strands can therefore be separated by breaking hydrogen bonds sequentially. The enzymatic splitting of these hydrogen bonds is important during the replication of the DNA molecule during cell division.

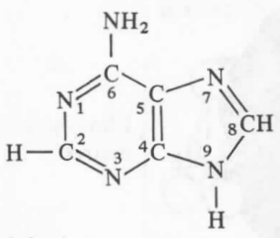
The double-stranded DNA exists in the form of a helix in which one strand is wound around the other in a precise manner (Fig. 2-17). The preciseness is due to the hydrogen bonding between specific bases on opposite strands. The two chains are complementary only when they are antiparallel. If you look at Figure 2-16 you will notice that one strand appears to be upside down. One strand has a free hydroxyl group attached to carbon 3 of the sugar, while in the opposite strand the free hydroxyl is on carbon 5 of the sugar. Each strand of DNA, therefore, has a 3′ and a 5′ end. The genetic material in prokaryotes is usually a double-stranded DNA molecule in which the ends are joined together to form a closed circle (Fig. 2-18). No other macromolecules are associated with prokaryotic DNA, but in eukaryotes the genetic material is surrounded by proteins called *histones*. The hereditary material, whether complexed with proteins or not, is referred to as a *chromosome*. Each chromosome consists of a single DNA molecule that is folded into a highly compact unit.

STRUCTURE OF RIBONUCLEIC ACID

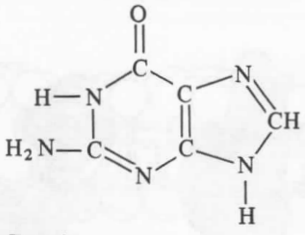
Ribonucleic acid (RNA) is a single-stranded linear molecule (a few viruses possess double-stranded RNA) in both prokaryotes and eukaryotes* and is similar to single-stranded DNA. RNA differs from DNA in that ribose is substituted for deoxyribose and the pyrimidine uracil is substituted for thymine (see Fig. 2-15). RNA exists in greater concentration in the cell than DNA and is divided into various types depending on its function in the cell. Each type is synthesized in the cell as a product of the

*The biological world is divided into prokaryotes and eukaryotes based primarily on the presence (eukaryote) or absence (prokaryote) of a nuclear membrane. See Chapter 4 for more detail.

Fig. 2-15. Structure of the components of the DNA molecule. A. Purines: adenine and guanine. B. Pyrimidines: cytosine, thymine, and uracil. C. Sugars: D-ribose and D-2-deoxyribose. D. Phosphate. E. Nucleotides: cytosine 5-monophosphate and adenosine 5-monophosphate.

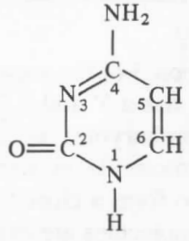


Adenine

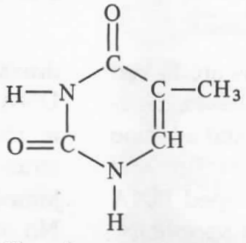


Guanine

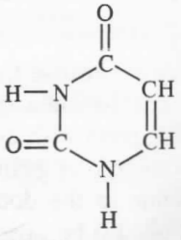
A



Cytosine

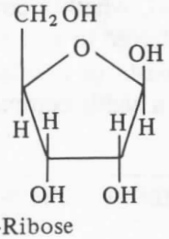


Thymine

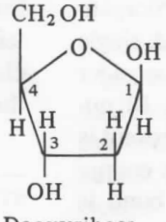


Uracil

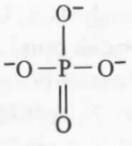
B



D-Ribose

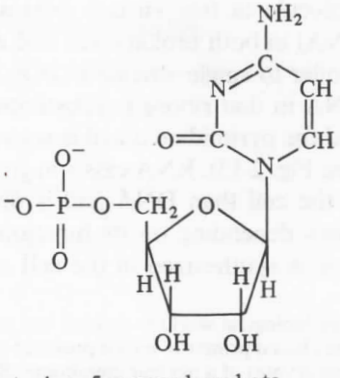


D-2-Deoxyribose

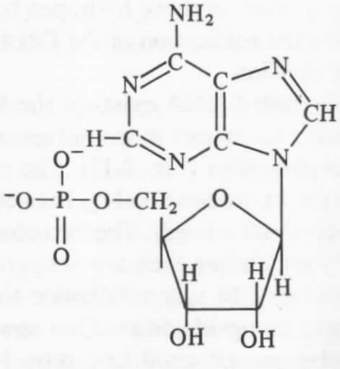


Phosphate

C D



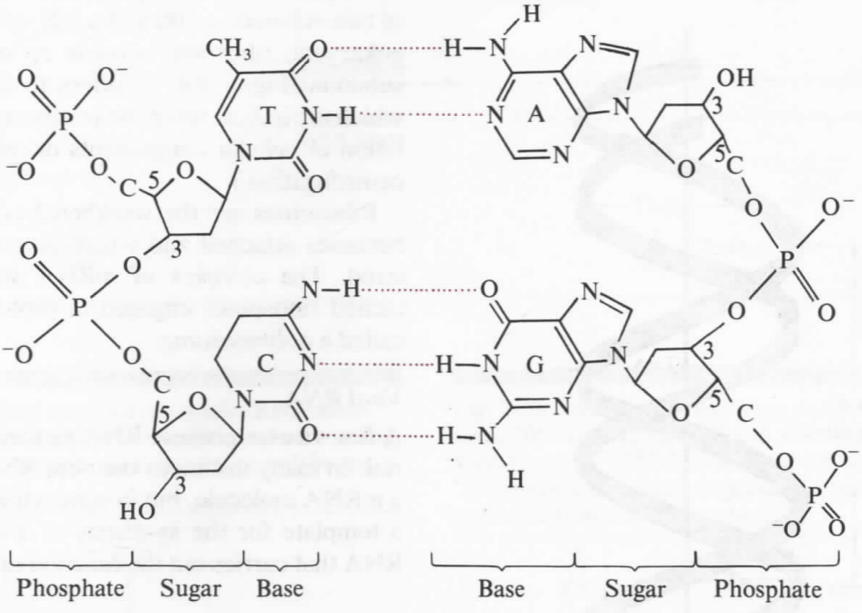
Cytosine 5-monophosphate



Adenosine 5-monophosphate

E

Fig. 2-16. Base pair arrangement between adjacent nucleotides on a portion of the DNA molecule. The pairing between thymine on one strand and adenine on the other is by two hydrogen bonds, while three hydrogen bonds bind guanine and cytosine. Note that the sugar groups on the right-hand chain appear to be upside down; actually the chains are antiparallel.



transcription of specific sequences on the DNA molecule.

Messenger RNA

The sequence of bases in the messenger RNA (mRNA) molecule is complementary to specific genetic sequences in the DNA molecule called *genes*. As you will discover in Chapter 5, the DNA molecule contains thousands of genes that can be transcribed into complementary mRNA molecules. If, for example, part of a DNA sequence is AAGCA, then the complementary part of the mRNA sequence will be UUCGU. The length of the DNA sequence that gives rise to a mRNA molecule may be from 100 to 4000 nucleotides, whereas the entire DNA molecule may be 1×10^7 nucleotides in length. The mRNA molecule is very unstable and in microbial cells has a half-life of only a few seconds. The function of the mRNA is to translate the information encoded in the DNA molecule into the amino acid sequences of proteins. This translation process takes place on cellular units called ribosomes (see Ribosomal RNA below).

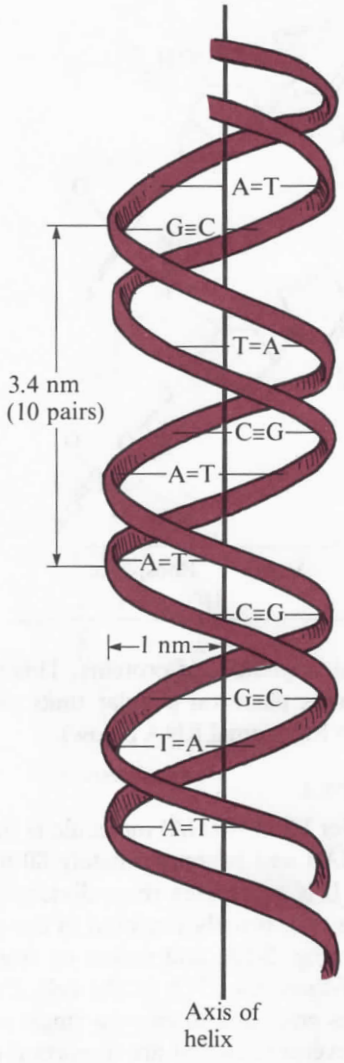
Transfer RNA

The transfer RNA (tRNA) molecule is the shortest of the RNA's and is approximately 80 nucleotides in length. It is a complex three-dimensional structure in the cell, usually depicted in the shape of a cloverleaf (Fig. 2-19), and makes up from 10 to 15 percent of the total RNA in the cell. Each tRNA molecule is equipped to carry a single amino acid and has several sites that are important during the synthesis of proteins. There are at least 20 different tRNA molecules to accommodate the 20 different amino acids found in protein molecules.

Ribosomal RNA

Ribosomal RNA (rRNA) makes up the bulk (70 percent) of the RNA in the cell and is complexed

Fig. 2-17. Model of DNA molecule showing the interaction and helical nature of the two strands. Not all possible base pairs are indicated; for example, there are 10 base pairs per turn of helix. (A = adenine; T = thymine; G = guanine; C = cytosine.)



with proteins to form structural units called *ribosomes*. Many thousands of ribosomes are found in the cell; their number varies depending on the relative metabolic activity of the cell. The greater the rate of growth of a cell the greater the number of ribosomes. The prokaryotic ribosome is composed of two subunits, a 50S and a 30S subunit, while the eukaryotic ribosome is made up of 60S and 40S subunits (Fig. 2-20). (*S* refers to Svedberg units, which are indicators of the relative rate of sedimentation of cellular components or molecules during centrifugation.)

Ribosomes are the workbenches where mRNA becomes attached and where proteins are synthesized. The complex of mRNA with several attached ribosomes engaged in protein synthesis is called a *polyribosome*.

Viral RNA

A few viruses possess RNA as their genetic material. In many instances the viral RNA behaves like a mRNA molecule, but in some viruses the RNA is a template for the synthesis of a complementary RNA that carries out the function of mRNA.

CARBOHYDRATES

GENERAL CHARACTERISTICS

Carbohydrates are the most frequently encountered energy source for organisms, but in plants they are also the major structural component (cellulose). Plants are the principal source of carbohydrates, which are produced during the process of photosynthesis. Carbohydrates are also part of the structure of bacterial cell walls and have many implications in the disease process.

Carbohydrates are chemically defined as polyhydroxyaldehydes or ketones. They may be divided into three groups—monosaccharides, oligosaccharides, and polysaccharides—based on their hydrolysis products and the number of carbons in the molecule.

Fig. 2-18. Conversion of linear stranded DNA to circular form. The interconversion of these two forms of the molecule requires specific enzymes: Endonuclease breaks internal bonds of the circular form, and DNA ligase is used to convert the linear form to the circular form.

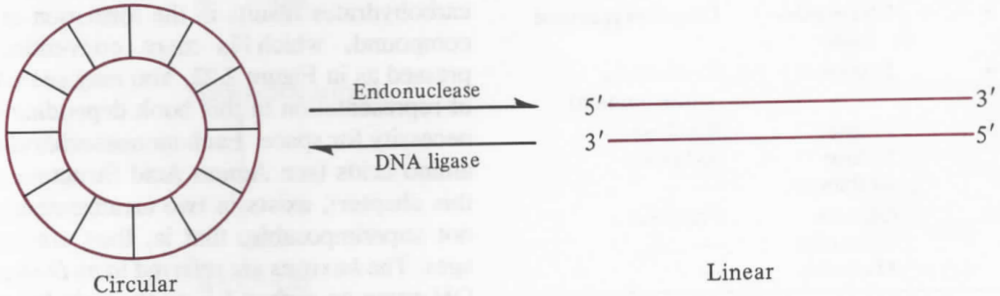


Fig. 2-19. Cloverleaf structure of transfer RNA molecule. 3′-OH end of molecule is site for covalent bonding of amino acid. A more detailed description will be presented in Chapter 5.

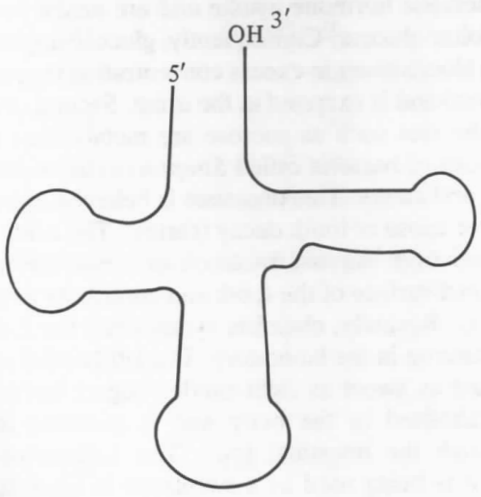


Fig. 2-20. Structure of ribosome. A. Prokaryotic ribosome 70S consisting of 50S and 30S subunits. B. Eukaryotic ribosome (80S) consisting of 60S and 40S subunits. "S" refers to sedimentation constant based on rate of sedimentation in an ultracentrifuge.

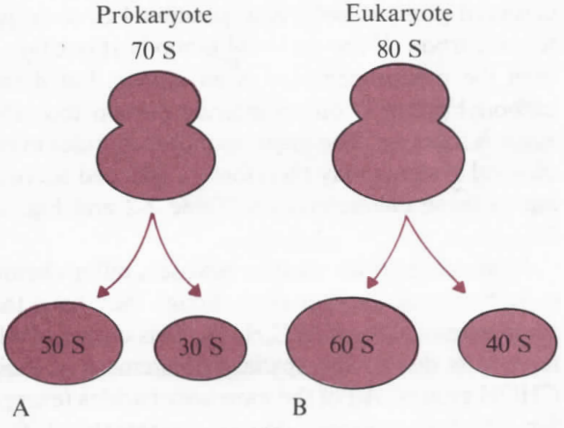


Table 2-2. Major Monosaccharides Found in Biological Systems*

Type	No. of carbons	Name of aldose	Name of ketose
Triose	3	Glyceraldehyde	Dihydroxyacetone
Tetrose	4	Erythrose	Erythrulose (uncommon)
Pentose	5	Ribose	Ribulose
		Xylose	Xylulose
		Arabinose	—
Hexose	6	Glucose	Fructose
		Galactose	—
		Mannose	—

*See Figure 2-21 for the structure of these monosaccharides.

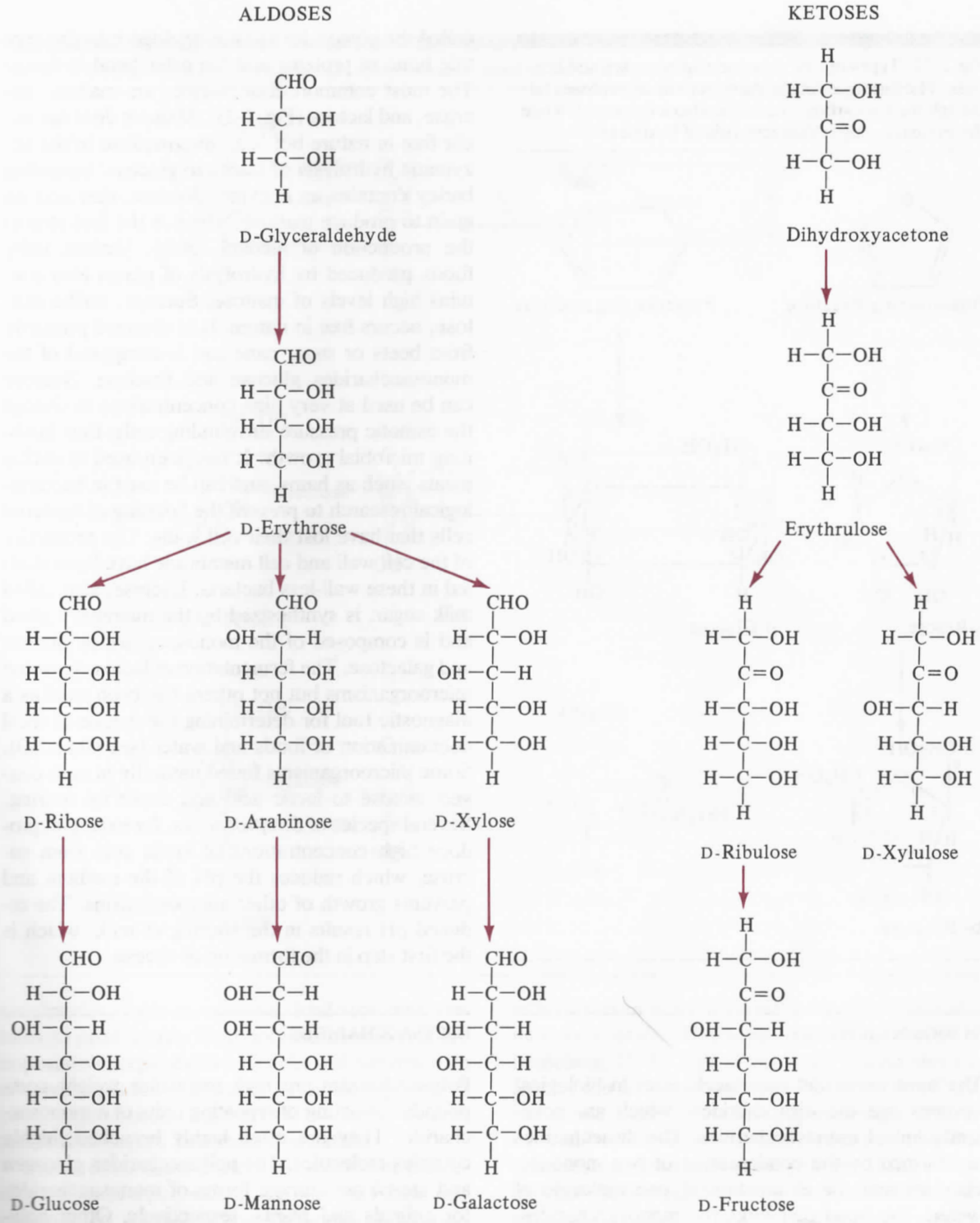
MONOSACCHARIDES

Monosaccharides are the simplest sugars (a sugar is a carbohydrate that is soluble in water and sweet to the taste) and have the empirical formula $C_nH_{2n}O_n$, where n may vary from 3 to 8. They can be further subdivided depending on whether the carbonyl group (C═O) is at position 1 or at an internal carbon. If the carbonyl group is at position 1 then the monosaccharide is an *aldose*, but if the carbonyl group is on an internal carbon then the sugar is a *ketose*. The major monosaccharides in biological systems may therefore be grouped according to these characteristics (Table 2-2 and Fig. 2-21).

Monosaccharides such as hexoses, differ chemically from one another even though they have the same empirical formula $C_6H_{12}O_6$. This chemical difference is due to the spatial arrangement of their CHOH groups. All of the monosaccharides (except for dihydroxyacetone) contain asymmetrical carbon atoms. Hexoses such as glucose, fructose, mannose, and galactose are all structural isomers of each other because of the relationship of the hydroxyl group attached to their asymmetrical carbon atoms. Carbons 2 through 5 of glucose and galactose are asymmetrical because each is bonded to four different groups, while on fructose only carbons 3 and 5 are asymmetrical (Fig. 2-21).

The conventional way of drawing the carbohydrates is in a straight chain with hydroxyl groups to the right or left. The intermolecular association between the aldehyde (H—C═O) and alcohol (H—C—OH) groups on five- and six-membered carbohydrates results in the formation of a cyclic compound, which is more conventionally expressed as in Figure 2-22. You may see either type of representation in this book depending upon the necessity for space. Each monosaccharide, as with amino acids (see Amino Acid Structure earlier in this chapter), exists in two arrangements that are not superimposable, that is, they are mirror images. The hexoses are referred to as *D-sugars* if the OH group on carbon 5 is on the right but as *L-sugars* if on the left. For pentoses the position of the OH group on carbon 4 determines whether the sugar belongs to the D or L family. The D-sugars are found normally in biological systems and are the ones metabolized by all types of organisms. There are situations in which D-sugars can be harmful. First, diabetics have a deficiency of the pancreatic hormone insulin and are unable to metabolize glucose. Consequently, glucose appears in the bloodstream in excess concentration (hyperglycemia) and is excreted in the urine. Second, sugars in the diet such as sucrose are metabolized by a species of bacteria called *Streptococcus mutans* in the oral cavity. This organism is believed to be the major cause of tooth decay (caries). The acids produced from sucrose metabolism break down the enamel surface of the tooth and contribute to tooth decay. Recently, chemists synthesized the L-form of sucrose in the laboratory. The left-handed sugar is just as sweet as right-handed sugars but is not metabolized in the body and is excreted intact through the intestinal tract. The L-form of the sugar is being used as a sweetener in some foods and thus has the potential for overcoming some of the health hazards associated with the D-sugars.

Fig. 2-21. Structure of two groups of monosaccharides, aldoses and ketoses, found in biological systems.


ALDOSES
KETOSES
D-Glyceraldehyde
Dihydroxyacetone
D-Erythrose
Erythrulose
D-Ribose
D-Arabinose
D-Xylose
D-Ribulose
D-Xylulose
D-Glucose
D-Mannose
D-Galactose
D-Fructose

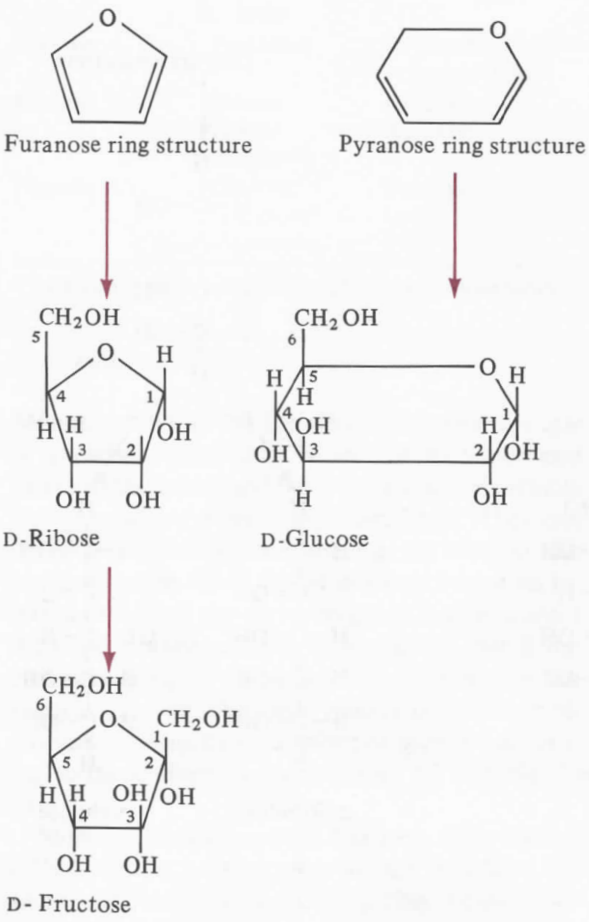


Fig. 2-22. Types of ring structures of pentoses and hexoses. The furanose ring is characteristic of pentoses (although the six-carbon fructose is also a furanose), while the pyranose ring is characteristic of hexoses.

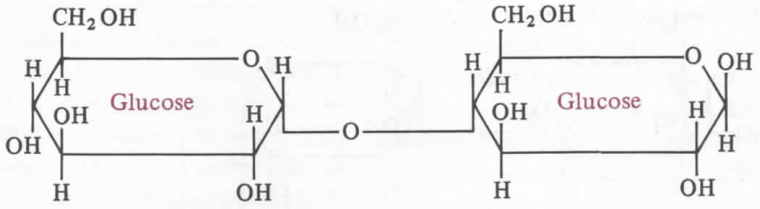
OLIGOSACCHARIDES

The most important oligosaccharides in biological systems are the disaccharides, which are covalently linked monosaccharides. The disaccharides are formed by the condensation of two monosaccharides and the elimination of one molecule of water. The bond that links the monosaccharides, called the *glycosidic bond*, is analogous to the peptide bond in proteins and the ester bond in lipids. The most common disaccharides are maltose, sucrose, and lactose (Fig. 2-23). Maltose does not occur free in nature but is an intermediate in the enzymatic hydrolysis of starch to glucose. Sprouting barley contains an enzyme, diastase, that acts on grain to produce maltose, which is the first step in the production of alcohol (malt). Various baby foods produced by hydrolysis of grains also contains high levels of maltose. Sucrose, unlike maltose, occurs free in nature. It is obtained primarily from beets or sugar cane and is composed of the monosaccharides glucose and fructose. Sucrose can be used at very high concentrations to change the osmotic pressure surrounding cells, thus inhibiting microbial growth. It has been used in curing meats, such as hams, and can be used in bacteriological research to prevent the bursting of bacterial cells that have lost their cell walls. The properties of the cell wall and cell membrane have been studied in these wall-less bacteria. Lactose, also called milk sugar, is synthesized by the mammary gland and is composed of the monosaccharides glucose and galactose. The fermentation of lactose by some microorganisms but not others has been used as a diagnostic tool for determining the degree of fecal contamination of foods and water (see Chap. 23). Some microorganisms found naturally in milk convert lactose to lactic acid and cause its souring. Several species of *Streptococcus*, for example, produce high concentrations of lactic acid from sucrose, which reduces the pH of the medium and prevents growth of other microorganisms. The reduced pH results in the souring of milk, which is the first step in the formation of cheese.

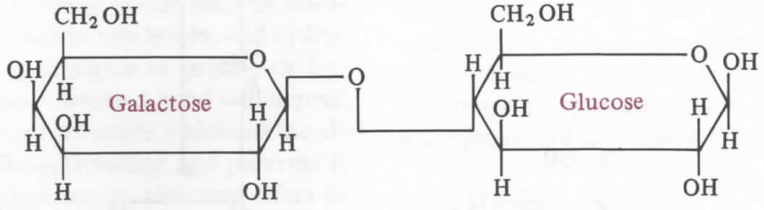
POLYSACCHARIDES

Polysaccharides are high molecular weight compounds consisting of repeating units of a monosaccharide. They are often highly branched, highly complex molecules. The polysaccharides *glycogen* and *starch* are storage forms of monosaccharides for animals and plants, respectively. Other poly-

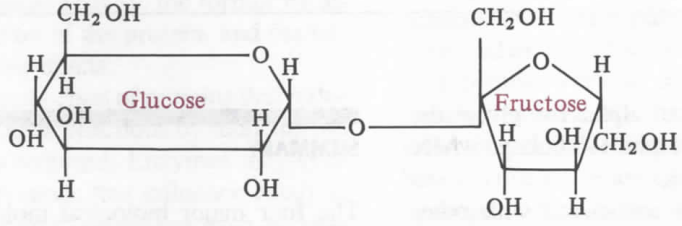
Fig. 2-23. Cyclic structure of the principal disaccharides found in biological systems.



MALTOSE



LACTOSE

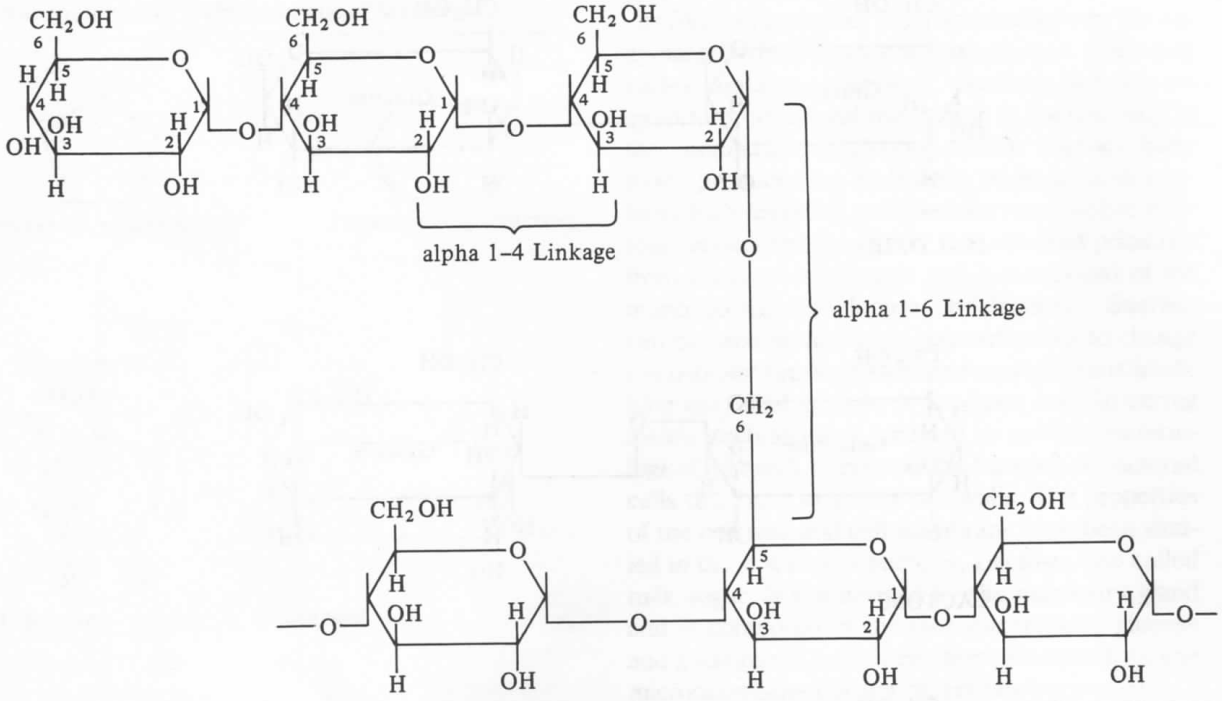


SUCROSE

saccharides, such as *cellulose*, lend structural stability to plant tissue. Most polysaccharides that are important energy reserves for the cell are made up of repeating units of glucose, but repeating units of other sugars, such as galactose, mannose, xylose, and arabinose, are also present in nature.

Unlike other macromolecules, the size or molecular weight of a polysaccharide may vary from one molecule to another. Polysaccharides show individuality even when they are made up of the same monosaccharide. This is due to (1) the degree of branching, (2) the type of glycosidic linkage (for example, the linkage may be from carbon 1 of one sugar unit to carbon 2 of the adjacent sugar unit, or from carbon 1 to carbon 3) and (3) the steric (spatial relationships of atoms) configuration at the asymmetrical carbon atoms, which may be described as either alpha or beta. The polysaccharide amylopec-

Fig. 2-24. Structure of a branched polysaccharide (amylopectin). The chain consists of alpha 1–4 linkages with alpha 1–6 branch points.



tin, for example, consists of alpha 1–4 glycosidic linkages interrupted by an alpha 1–6 linkage where branching occurs (Fig. 2-24).

Polysaccharides may be associated with other macromolecules to form complexes. Lipopolysaccharides are characteristic of the cell wall of certain bacteria and form a component that is toxic to mammalian tissue systems. As discussed previously, the major cell wall component that lends structural support to prokaryotic cells is a layer (the peptidoglycan layer) consisting of alternating molecules of N-acetylglucosamine and N-acetylmuramic acid in a beta 1–4 linkage in which the chains are linked by small peptide units (see Fig. 4-13).

SUMMARY

The four major biological molecules are protein, lipids, nucleic acids, and carbohydrates. Each plays an important role in the structure and function of living cells as well as viruses.

PROTEINS

Proteins are made up of monomers called amino acids that are linked via peptide bonds. Proteins have several functions in the cell: (1) as enzymes that catalyze biological reactions, (2) as structural components of the cell, (3) in immunological reactions when they act as antigens, (4) as contractile fibers, (5) as toxins, and (6) as transport proteins.

Protein structure can be described at four levels: primary, secondary, tertiary, and quaternary. Primary structure refers to the sequence of amino acids in the protein, which in turn influences the conformation of the molecule. Secondary structure refers to the manner in which the polypeptide is bent and folded. The conformation of the protein may take three different forms: random coil, pleated sheet, and alpha helix. The alpha helix is the most commonly encountered form and results from the hydrogen bonding between the C═O of one amino acid and the N—H bond three amino acids down the chain. The tertiary structure refers to the interaction between the specific R groups on the polypeptide and involves bonds such as disulfide bonds, hydrogen bonds, salt bonds, and hydrophobic forces. The appearance of proline or hydroxyproline determines where a bend will appear in the helix. The tertiary structure maintains the alpha helix in a stable conformation and prevents it from unfolding. The quaternary structure refers to the interaction between two or more polypeptides when a protein is made up of individual polypeptide subunits.

Denaturation and hydrolysis can cause the protein to lose its biological activity, the former by altering the conformation of the protein, and the latter by breaking peptide bonds.

Enzymes are a special class of proteins that influence the rate of biological reactions by reducing the energy of activation required. Enzymes may contain additional components that influence catalytic activity, including organic molecules such as vitamins and inorganic ions such as metals. Enzymes possess a site (active site) that is specific for the substrate, and this specificity may be limited to one type of molecule or to several molecules with specific functional groups. Enzymes are affected by many chemical and physical factors including pH, temperature, substrate concentration, and inhibitors. Some microbial enzymes have been extracted and used in clinical situations.

LIPIDS

Lipids are substances that are soluble in organic solvents but insoluble in water. Lipids may be simple or compound, but both consist of fatty acids in ester linkage. Simple lipids are esters of fatty acids with varying alcohol groups, the most important of which is glycerol. Compound lipids contain additional elements such as phosphorous, nitrogen, or sulfur, or in some cases they may be complexed with macromolecules such as protein (lipoproteins). Lipids perform three major functions in the cell: (1) They are a source of energy, (2) they are structural components of cell wall and cell membranes, and (3) they form a protective structure around some viruses. The hydrophobic and hydrophilic nature of some lipids make them ideal for membrane components in which hydrophobic and hydrophilic molecules must be transported.

NUCLEIC ACIDS

Nucleic acids are the carriers of the genetic information of the cell and consist of long chains of repeating units called nucleotides. Each nucleotide consists of a sugar phosphate plus a nitrogenous base, either a purine or a pyrimidine. The sugar is a pentose—ribose in ribonucleic acid (RNA) and deoxyribose in deoxyribonucleic acid (DNA). DNA is a double-stranded molecule in which the bases in one chain are complementary to, and form hydrogen bonds with, the bases on the opposite chain. DNA is the hereditary material in all microorganisms and in most viruses. RNA is the hereditary material in only a few viruses. One of the major differences between prokaryotic and eukaryotic DNA is that the latter is associated with proteins called histones while the former is not. Ribonucleic acids perform several functions in the cell: messenger RNA (mRNA) is complementary to one strand of DNA and carries information from DNA into the cytoplasm, transfer RNA (tRNA) carries amino acids that are to be engaged in protein synthesis, and ribosomal RNA (rRNA) makes up part of the components called ribosomes, which serve as the workbenches for protein synthesis.

CARBOHYDRATES

Carbohydrates are polyhydroxyaldehydes or ketones that function as energy sources and structural components in the cell. They are divided into three groups—monosaccharides, oligosaccharides, and polysaccharides—depending on the number of sugar units. Monosaccharides are the simplest sugars, containing three to eight carbons. The hexoses (sugars with six carbons) are the most commonly found monosaccharides in the cell. They all have the same empirical formula but differ in the spatial arrangement of their hydroxyl groups. Oligosaccharides may contain from two to ten monosaccharide units, but the most important type is the disaccharides, which consist of two covalently bonded monosaccharides. Sucrose, lactose, and maltose are among the most important disaccharides. Polysaccharides are high molecular weight (up to 1,000,000 daltons) carbohydrates consisting of repeating units of monosaccharides. They are important as storage forms of energy (starch and glycogen) as well as structural components (cellulose) in cell wall and cell membranes.

QUESTIONS FOR STUDY

1. Describe those forces or bonds that are important in the primary, secondary, tertiary, and quaternary structure of proteins.
2. Name at least five functions that proteins perform in the cell.
3. How does one distinguish between L- and D-amino acids? D- and L-sugars? Which forms are more common for each molecule in biological systems?
4. Are all enzymes made up of subunits? What might be the importance of having two or more subunits in a protein molecule?
5. What is the distinction between competitive and noncompetitive inhibition in terms of site of action on the enzyme?
6. How are enzymes capable of lowering the energy of activation for biological molecules?
7. What makes phospholipids important in the construction of biological membranes?
8. Name some compound lipids that are important in biological membranes.
9. What functions do RNA molecules play in the cell?
10. What is the ratio of purines to pyrimidines in the DNA molecule? In the RNA molecule?
11. How are DNA and RNA different? How are they similar?
12. List some of the more common oligosaccharides, monosaccharides, and polysaccharides found in biological systems.
13. Draw the straight-chain and cyclic forms of the sugar glucose.

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3. OBSERVATION OF MICROORGANISMS: MICROSCOPY AND STAINING

OBJECTIVES

To list the advantages and disadvantages of the following microscopes: compound bright-field, dark-field, phase-contrast, fluorescent, transmission electron microscope, and scanning electron microscope

To describe what is meant by resolving power and the factors affecting it

To describe the techniques of thin sectioning, negative staining, and freeze-etching as they apply to election microscopy

To differentiate between a simple and differential stain

To describe the steps in the Gram stain and the significance of what is meant by gram-positive and gram-negative

To define: acid-fast stain, hanging drop technique, immunofluorescence, fluorochrome, immersion oil, refractive index

Microscopy
- Resolving Power
- Types of Microscopes
  - Compound Bright-Field
  - Dark-Field
  - Phase-Contrast
  - Nomarski Interference
  - Fluorescent
  - Electron
    - Transmission Electron Microscopy
    - Scanning Electron Microscopy
- Preparation of Live Specimens: The Hanging Drop Technique

Staining
- Simple Stain
- Differential Stains
  - *Gram Stain*
  - *Acid-Fast Stain*
- Negative Stain
- Special Stains

Probably one of the more exciting aspects of microbiology for the beginning student is the realization that individual microorganisms, as small as they are, have shape and are composed of structures that can be identified. Visual identification of microorganisms is impossible with the naked eye because of their small size (Fig. 3-1 shows the relative sizes of various groups of microorganisms and viruses); however, bacterial images can be magnified with the aid of a microscope. Most of the time, because of their transparency, microorganisms must be stained to enhance their visualization.

The purpose of this chapter is to discuss the various types of microscopes that are used in the research and clinical laboratory to identify and characterize microorganisms and viruses. In addition we will also discuss the primary staining techniques used in the clinical laboratory.

MICROSCOPY

RESOLVING POWER

One of the purposes of a microscope is to enlarge or magnify the image, but magnification is of no value if the image is blurred. It is therefore important that the microorganism is clearly defined when observed. The property of definition is called the *resolving power*, and it is this property that determines the quality of the microscope. The resolving power of a microscope refers to the smallest detectable separation of two points or objects. In other words, how close can two objects be brought together and still be seen as two clearly defined objects? The resolving power of a microscope is dependent on the wavelength of light used in the optical system as well as a characteristic of the objective lens called the *numerical aperture*. The formula for resolving power (RP) is

$$RP = \frac{\text{wavelength}}{2 \times \text{numerical aperture}}$$

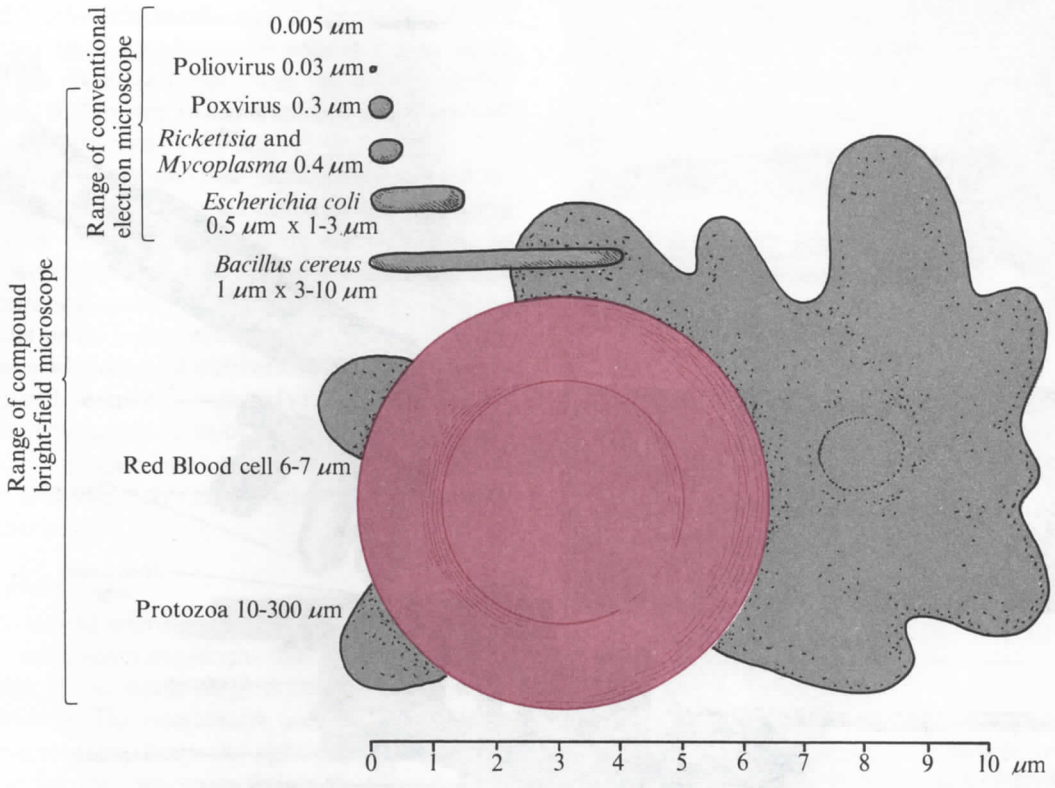
Since both the wavelength and the numerical aperture have narrow limits, the resolving power of the microscope using conventional light sources is very limited. Only with the advent of the electron microscope has it been possible to increase greatly the magnification and, more important, the resolving power of optical instruments.

TYPES OF MICROSCOPES

Compound Bright-Field

The compound bright-field microscope (Fig. 3-2) is called compound because the total magnification is

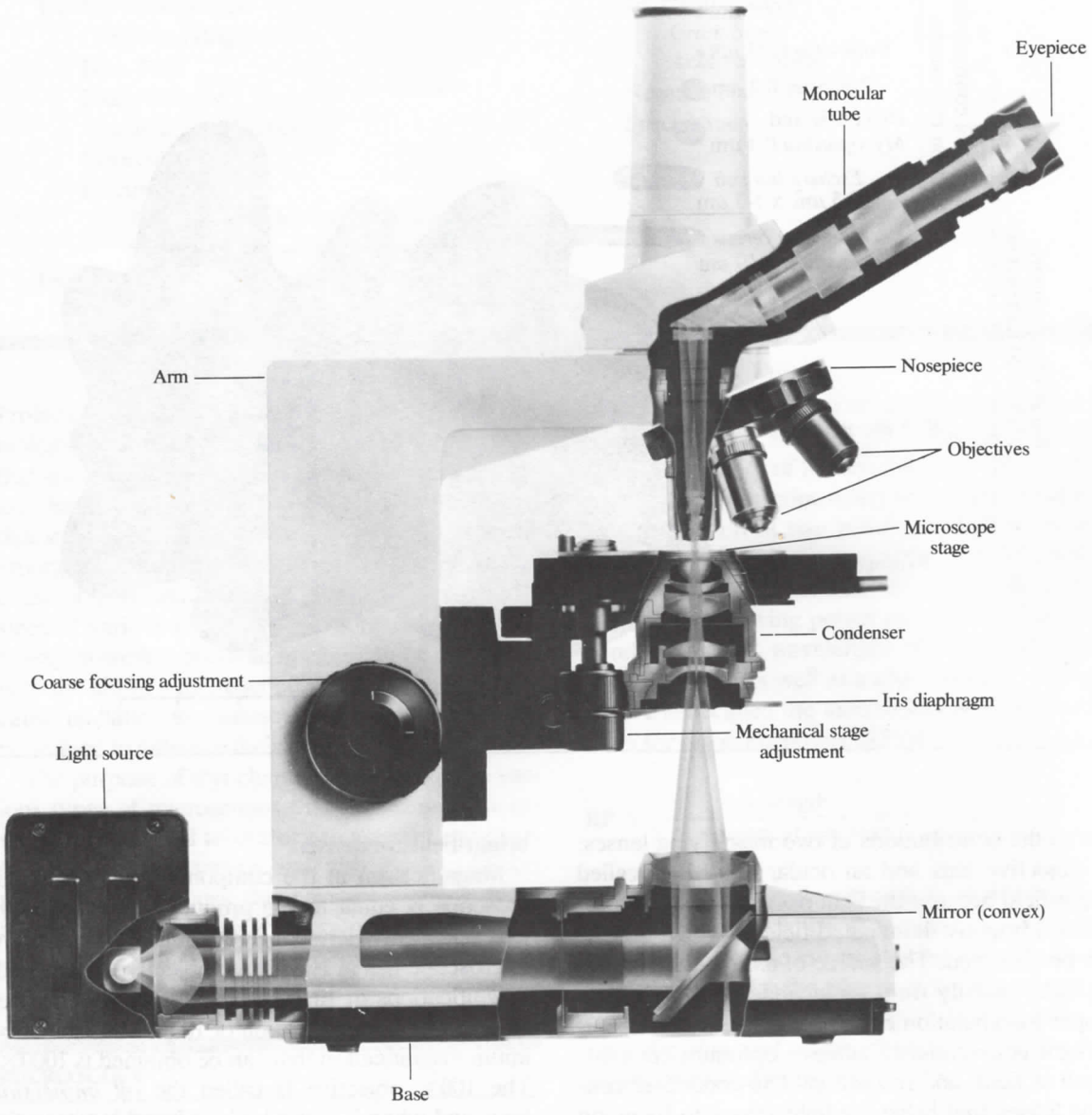
Fig. 3-1. Comparative size of microorganisms.



due to the contributions of two magnifying lenses: an objective lens and an ocular lens. It is called bright-field because the light passing through it produces a brightly illuminated field in which objects can be observed. The source of illumination is visible light, usually from an incandescent bulb. The proper manipulation of the condenser and iris diaphragm is essential to achieve optimum contrast, depth of field, and resolution. The condenser contains lenses that bring the light rays into focus on the specimen, while the iris diaphragm, located between the condenser and the light source, controls the amount of light entering the condenser. The background surrounding the specimen will therefore appear very bright using the conventional bright-field condenser.

Magnification in the compound bright-field microscope is equal to the product of the objective lens and ocular lens magnifications. The standard microscope has at least three objective lenses with magnifications of 10×, 44×, and 100×. The ocular lens has a magnification of 10×. Thus the maximum magnification that can be obtained is 1000×. The 100× objective is called the *oil immersion lens*, and when it is used oil is placed between the coverslip covering the specimen and the lens. By using the oil immersion lens with the 100× objective the mathematical expression for the numerical aperture can be increased, thereby increasing the resolving power of the microscope. Immersion oil

Fig. 3-2. Compound bright-field microscope. (Courtesy Nikon, Inc., Rochester, NY.)



has the same refractive index* as the glass slide and therefore light rays are not further refracted as they would be in air. The resolving power of the compound bright-field microscope is approximately 0.2 μm; that is, if two bacterial cells 0.2 μ or more apart are examined each can be clearly distinguished. If they are closer than 0.2 μm, they will appear as a blurred single cell.

Conventional bright-field illumination will not reveal brightness differences between the structural details of the specimen and its surroundings. In other words the bacteria appear transparent. The transparency problem can be rectified by staining the cells or by using other microscopic techniques that take advantage of differences in optical density and refractive index, produced in the specimen.

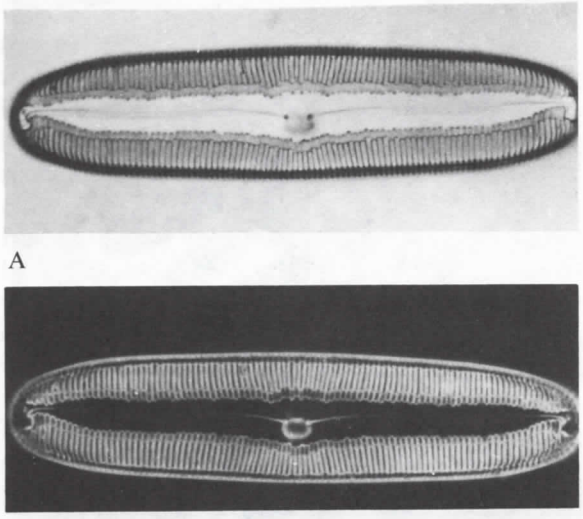
One of the advantages of bright-field microscopy is that living specimens can be examined. For example, it is relatively easy to determine the motility of bacteria.

Dark-Field

The dark-field microscope is frequently used to observe viable microorganisms that are very small or thin and whose morphology is best observed without staining. The condensers are the key to dark-field microscopy. A special light condenser is substituted for the customary bright-field condenser. Dark-field condensers channel the light rays at such an oblique angle that undiffracted rays are not collected by the objective lens. Only the rays diffracted by the specimen on the slide enter the objective. The background surrounding the specimen appears dark or black (Fig. 3-3). Organisms or structures whose diameter is less than the resolving power of the compound microscope can be observed by this technique. Bacterial flagella, which are only 0.02 μm in diameter, can be observed because they scatter enough light for their profile to be seen. Spirochetes, whose width is between 0.15 μm and 0.2 μm, can also be easily observed using dark-field microscopy (Fig. 3-4).

*Refractive Index (RI) is a measure of the extent to which light is slowed down by a medium. Oil has the same RI as glass, but air has a smaller RI than glass.

Fig. 3-3. Comparison of the images produced by (A) compound bright-field microscopy and (B) dark-field microscopy. (From R. F. Smith, *Microscopy and Photomicrography*. New York: Appleton-Century-Crofts, 1982.)



A

B

Fig. 3-4. Dark-field microscopy of spirochetes (the spiral-shaped microorganisms). (Courtesy Centers for Disease Control, Atlanta, GA.)

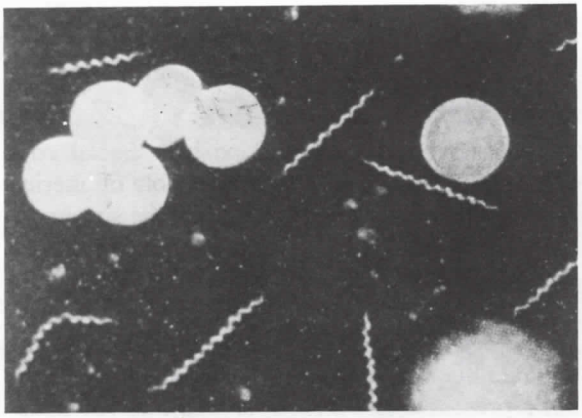
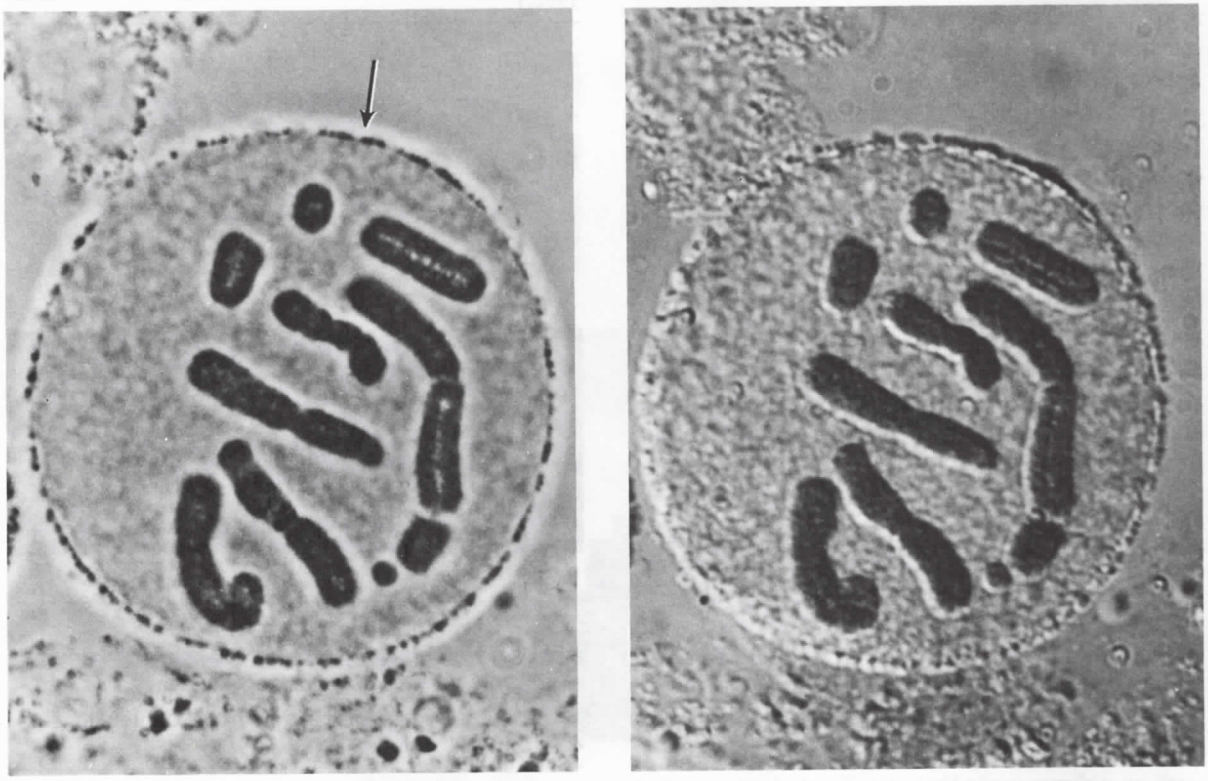


Fig. 3-5. A. Phase-contrast microscopy. Note the halo (*arrow*) around the spherical nucleus. B. Nomarski interference microscopy. Note that Nomarski interference microscopy appears to provide greater definition or clarity than does phase-contrast. (From R. F. Smith, *Microscopy and Photomicroscopy*. New York: Appleton-Century-Crofts, 1982.)



A B

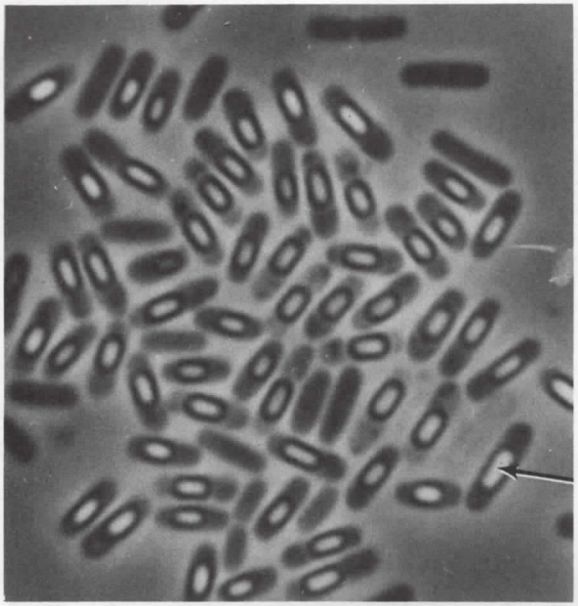
Phase-Contrast

The phase-contrast microscope has special condensers and objectives that are capable of altering light as it passes through and around the specimen. Light can be diffracted not only by the cell wall but also by any large structures in the cytoplasm whose density is greater than the surrounding medium. Every object observed by this technique is surrounded by a halo of light (Fig. 3-5A), which does not represent a real structure. The internal structure of the cell can be revealed by this technique. For example, nuclear bodies and endospores (Fig. 3-6) are easily observed.

Nomarski Interference

Nomarski interference microscopy is similar in many respects to phase-contrast microscopy. This technique requires specimens that are thicker than bacteria, for example, vegetative cells and spores of fungi. The presence of a polarizer and special prisms in the condenser result in the formation of a clearer image than that obtained with phase-contrast. The object, for example, has no halo and there is a three-dimensional appearance to the specimen (Fig. 3-5B).

Fig. 3-6. Phase-contrast micrograph of bacterial cells in which endospores (*arrow*) have been produced. (From A. Rosenbluh, et al., *J. Bacteriol.* 148:341, 1981.)



Fluorescent

Many materials are luminescent, that is, they absorb energy, some of which is released as light instead of heat. If the substance absorbs a quantity of light and, after a delay of less than one-millionth of a second, re-emits a quantity of light of a larger wavelength, the substance is called *fluorescent*. A few compounds fluoresce when illuminated with ultraviolent light or other short wavelengths of light; these are called *fluorochromes*. Microbiological material can be studied by coating it with fluorochromes. An important type of fluorescent microscopy is called *immunofluorescent* microscopy. Fluorochromes may be conjugated to specific antibodies to identify unknown specimens or structures (Fig. 3-7), or they may be conjugated to specific antigens to detect antibodies. This technique has great clinical applications. An unknown clinical specimen, for example, can be treated with a known conjugated antibody. If the antibody is specific for an antigen on the clinical specimen, a complex of antigen, dye, and antibody is formed. The complex is then exposed to wavelengths of 350 nm

Fig. 3-7. Technique for producing fluorescent antibodies. Bacterial capsule (composed of a polysaccharide that appears here as a ring surrounding the cell wall) is removed and injected into rabbit. Antibodies to capsule are produced in the rabbit and then removed. A fluorochrome is conjugated to the antibodies, which will bind to organisms possessing specific capsule. Organism will be seen to glow when observed microscopically.

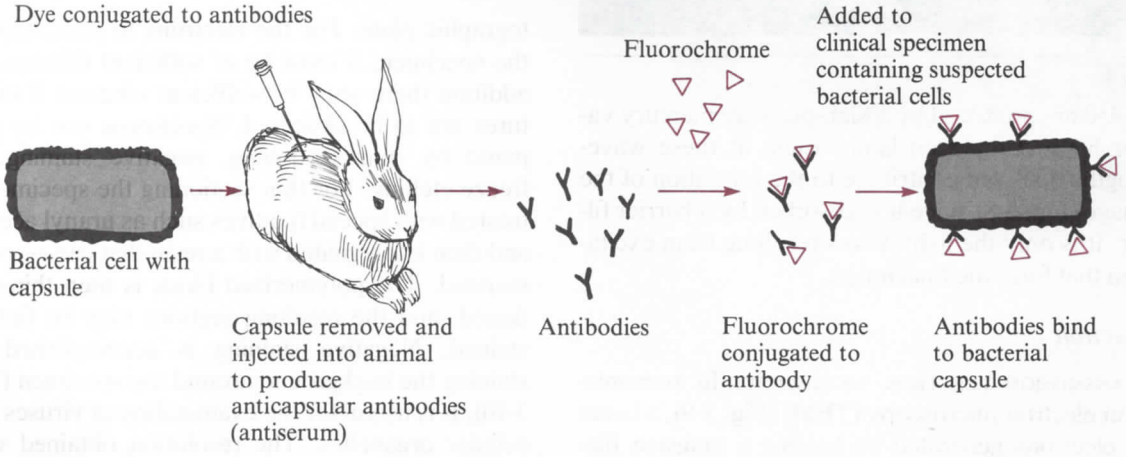
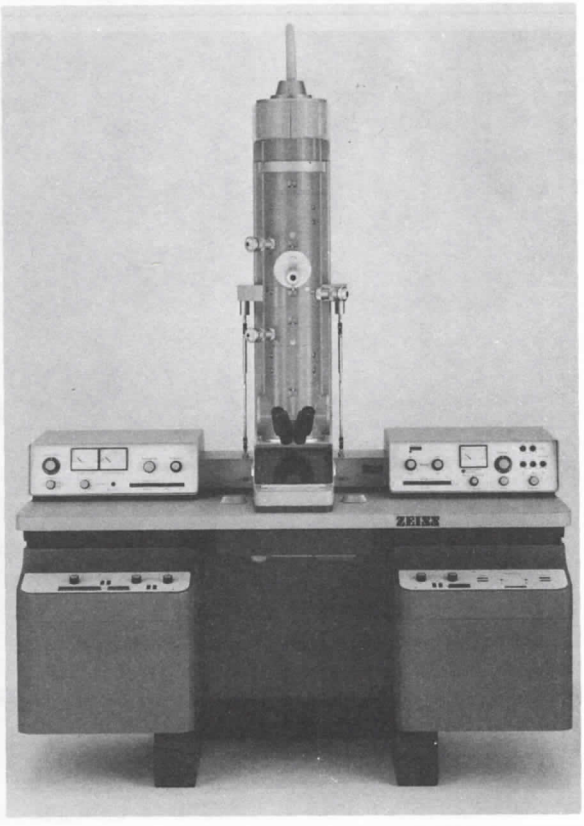


Fig. 3-8. Photomicrograph of a rod-shaped microorganism labeled by indirect fluorescent antibody technique using rabbit antiserum. (From W. W. Barrow and P. J. Brennan, *Infect. Immun.* 36:678, 1982.)

Fig. 3-9. Transmission electron microscope. (Courtesy Carl Zeiss, Inc., New York, NY.)

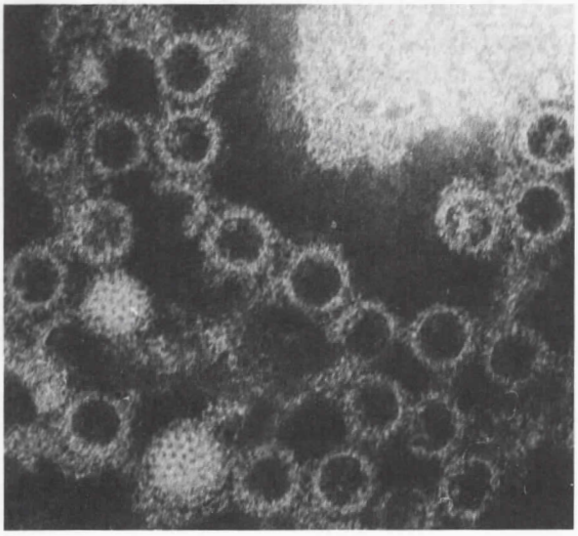


to 450 nm produced by a high-pressure mercury vapor lamp or halogen lamp. Light at these wavelengths does not contribute to the formation of the image (Fig. 3-8) since it is absorbed by a barrier filter. It is only the light waves resulting from excitation that form the final image.

Electron

TRANSMISSION ELECTRON MICROSCOPY. In transmission electron microscopy (TEM) (Fig. 3-9), a beam of electrons generated by heating a tungsten filament is passed through a specimen and onto a photographic plate. For the electrons to pass through the specimen, it must be of sufficient thinness. In addition there must be sufficient contrast if structures are to be observed. Specimens can be prepared by thin sectioning, negative staining, or freeze-etching. For thin sectioning the specimen is treated with special fixatives such as uranyl acetate and then impregnated with a resin that can be polymerized. The polymerized block is then thin-sectioned, and the resulting sections may be further stained. Negative staining is accomplished by staining the background around the specimen (Fig. 3-10). It is useful for the examination of viruses and cellular organelles. The resolution obtained with the TEM is 0.5 nm (0.0005 μm); the magnifications

Fig. 3-10. Negative staining of virus viewed by transmission electron microscopy ($\times$ 200,000 before 30% reduction). (From T. Kimura and T. Murakami, *Infect. Immun.* 17:157, 1977.)



obtained may be as high as 200,000 $\times$. One advantage of using TEM is that internal structures can be observed because electrons penetrate the specimen. One of the disadvantages of TEM is that the image produced is two-dimensional. In addition the specimen must be dried and sectioned for examination, a technique that kills the cells and makes the observation of living organisms impossible.

SCANNING ELECTRON MICROSCOPY. In scanning electron microscopy (SEM) (Fig. 3-11), a beam of electrons is scanned over the surface of a metal-coated specimen. Secondary electrons are emitted and produce an image that may be observed directly or photographically recorded. The image produced is three-dimensional (Fig. 3-12), which is very useful when the relationship of surface structures is examined. The disadvantage is that resolution is limited to 0.02 μm and magnifications to 10,000 $\times$.

Fig. 3-11. Scanning electron microscope. (Courtesy Carl Zeiss, Inc., New York, NY.)

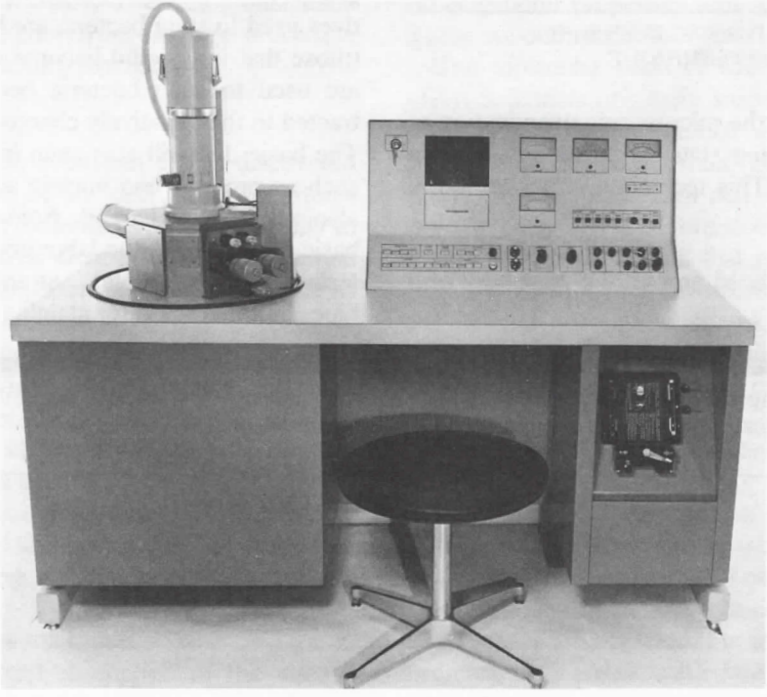
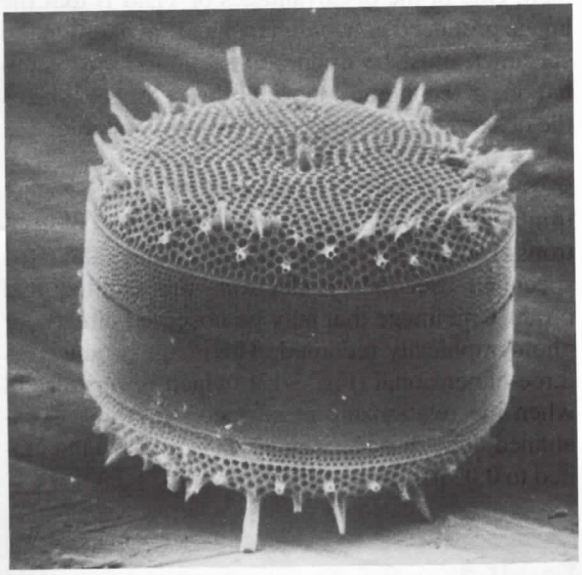


Fig. 3-12. Scanning electron micrograph of a freshwater diatom. (Courtesy F. E. Round.)



PREPARATION OF LIVE SPECIMENS: THE HANGING DROP TECHNIQUE

One technique for the microscopic examination of bacteria in the living state is called the *hanging drop preparation*. This technique is performed in the following way:

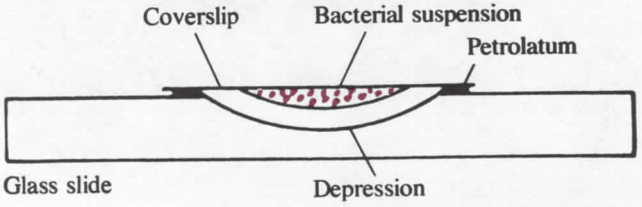
1. Petrolatum is placed around the periphery of a glass coverslip.
2. A drop of a bacterial suspension is transferred with an inoculating loop to the center of the coverslip.
3. A slide with a central depression is placed over the coverslip, and the preparation is inverted. The bacterial suspension will hang in the depression (Fig. 3-13) and can be observed under high power of the compound microscope.

The hanging drop technique is useful in determining the motility of bacteria and also gives an undistorted view of the morphology and arrangement of bacterial cells.

STAINING

Bacteria are difficult to observe because of their transparency. Therefore staining is an important aspect in the observation and identification of microorganisms. The microbial surface is negatively charged because of the presence of phosphate groups and carboxyl groups; therefore the types of dyes used to stain bacteria are limited. Basic dyes (those that ionize and become positively charged) are used to stain bacteria because they are attracted to the negatively charged bacterial surface. The basic dye will also stain internal components such as proteins and nucleic acids since they are also negatively charged. Some of the preferred basic dyes used in the laboratory for staining bacteria are crystal violet, safranin, and methylene blue. In preparation for staining procedures, a bac-

Fig. 3-13. The hanging drop preparation. The bacteria suspended in the concavity of the slide are free to move. Motility can be determined by observing bacterial movement with the high power of the compound microscope.



terial suspension is first smeared on the surface of a slide with an inoculating loop. The smear is air-dried and then heat-fixed to the slide. The slide is then ready for staining.

SIMPLE STAIN

In simple staining a suitable basic dye is applied to a heat-fixed smear for two or three minutes. The excess dye is washed off and the stained preparation observed microscopically.

DIFFERENTIAL STAINS

The chemical composition of many bacterial components is variable enough so that certain structural distinctions can be made between bacterial species or groups of bacteria. One way this objective can be accomplished is through the use of differential staining procedures, in which two stains are applied separately to a heat-fixed bacterial smear. The two most widely used differential staining procedures are the *Gram-staining* procedure and the *acid-fast staining* procedure.

Gram Stain

Some years ago Dr. Christian Gram discovered that bacteria could be divided into two groups based on the ability or inability of stained cells to resist decolorization by alcohol. The sequence and function of each component in Gram's stain are as follows:

1. Primary stain. The heat-fixed smear is stained with crystal violet, the primary stain. The excess stain is washed off the slide. The cells at this stage appear dark blue or purple.
2. Iodine. The crystal violet can be further fixed to the cell by the addition of a dilute solution of iodine that produces a crystal violet–iodine complex.
3. Decolorization. A solution of 95% alcohol (or alcohol and acetone) is applied to the stained smear. The alcohol dehydrates carbohydrates, which are present in large amounts in cell walls of gram-positive organisms. They, therefore, retain the stain (or stain-iodine complex). Walls of gram-negative organisms contain a high concentration of lipids, for which alcohol is a solvent. Pore sizes are presumably enlarged, and the dye complex escapes.
4. Counterstain. Safranin, a red dye, is the secondary stain applied to the fixed preparation. If the cells are decolorized with alcohol, they will take up the safranin and appear red (gram-negative). If the cells are not decolorized, the safranin will have no effect on the already-stained preparation and the cells will remain blue or purple (gram-positive).

Gram's stain is one of the first important identification procedures after isolation and allows one to divide the bacterial world into at least four categories: gram-positive cocci, gram-negative cocci, gram-positive bacilli, and gram-negative bacilli. When the Gram-staining characteristic of the cell has been determined, it is also possible to assign other general properties that apply to the group. These are outlined in Table 3-1.

The molecular basis of the Gram stain is not clear. Scientists originally suspected that the concentration of lipid in the cell wall determined the outcome of the Gram stain. Gram-negative cells possess more lipid in their cell walls than do gram-positive cells, and therefore alcohol produces pores in the gram-negative cell that result in loss of the primary stain. Other investigators proposed that the alcohol decolorizer shrank the gram-positive cell, preventing loss of the primary stain, but had no effect on the gram-negative cell.

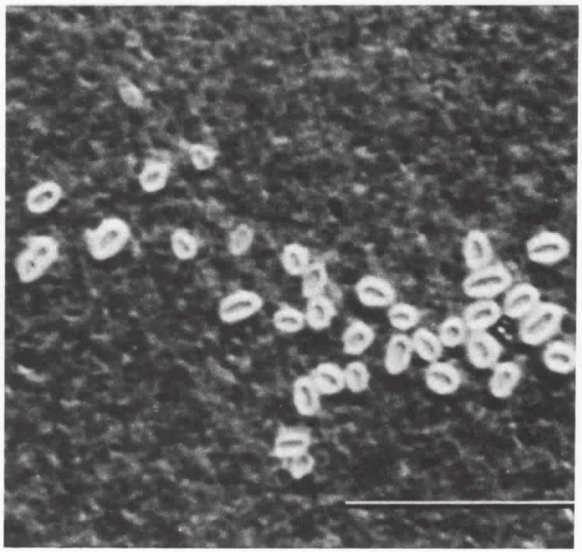
Acid-fast Stain

The cell surface components of some bacteria such as the genera *Mycobacteria* and *Nocardia* contain waxes and phospholipids that other bacteria do not contain. These bacteria are basically gram-positive if the waxes are removed, but in their natural state ordinary dyes cannot penetrate the waxy surface. These bacteria can be stained by the acid-fast tech-

Table 3-1. Characteristics of Gram-positive and Gram-negative Bacteria

Characteristic	Gram-positive	Gram-negative
Cell wall composition	Less complex; contains primarily peptidoglycan and very little lipid	More complex; little peptidoglycan but outer membrane with considerable lipid, polysaccharide, and protein
Penicillin sensitivity	More susceptible	Less susceptible
Resistance to sonic disruption and other physical measures	More resistant	Less resistant
Resistance to cell wall lytic enzyme lysozyme	Less resistant	More resistant
Dye inhibition	Inhibited by crystal violet	Not inhibited by crystal violet
Spore formation	Some bacilli are sporeformers	No bacilli are sporeformers
Toxin production	Toxin produced is an exotoxin	Toxin produced is usually an endotoxin

Fig. 3-14. Capsule-producing rod-shaped bacteria suspended in India ink and observed under oil immersion. Capsules appear as white halos around the bacteria. (From D. J. Politis and R. Goodman, *Appl. Environ. Microbiol.* 40:596, 1980.)



nique. Carbolfuchsin, a red dye, is applied to a heat-fixed smear of the organisms, which is then gently heated in a flame. The excess dye is removed by water, and the smear is treated with acid alcohol. In the final step a counterstain methylene blue is applied to the smear. Organisms that are acid-fast retain the carbolfuchsin, while non-acid-fast bacteria do not retain carbolfuchsin and appear blue.

NEGATIVE STAIN

By staining the background of a bacterial specimen and not the cells, one can obtain what appears to be a dark-field image. India ink or nigrosin is added to the microbial suspension, which is then spread on a microscope slide, dried, and observed microscopically. This technique is valuable in determining the presence of capsules on some bacteria (Fig. 3-14).

SPECIAL STAINS

Other staining techniques are available to the technologist but are not as routinely used as the ones previously mentioned.

1. Flagella stain. The flagella stain requires a great deal of laboratory skill and is not routinely performed by the technologist. The determination of the arrangement of flagella on the bacterial surface is sometimes useful in the classification of species.
2. Spore stain. If a bacterium produces a spore, identification is made easier by the fact that tests for nonsporulating bacilli can be eliminated. Even the placement of the spore within the cell is of value in identifying species within a genus.
3. Metachromatic granule stain. Some microorganisms accumulate phosphates in the cell in the form of granules called *metachromatic granules* (also called *volutin*). These granules can be selectively stained to contrast with the cytoplasm.

SUMMARY

Microscopy is a science dealing with the observation of magnified images. Magnification of microorganisms is an important aspect of laboratory identification, and the ability to define the magnified image (i.e., resolving power) is a critical feature of the microscope lens system. Various types of microscopes with different magnifications and resolving power are available. Most of the conventional microscopes used in the clinical laboratory have maximum magnifications approaching 1000× with resolving powers of approximately 0.2 μm. They differ primarily in the way the specimen is illuminated. The compound bright-field microscope illuminates the specimen in such a way that the background appears bright in contrast to the darker image of the specimen. Bright-field microscopy is useful for examining not only fixed specimens but also live specimens. The dark-field microscope uses a special condenser to make the background dark and the specimen bright. Phase-contrast microscopy employs special condensers and objectives so that density variations in the structures of the cell can be observed. Nomarski interference microscopy is similar to phase-contrast except that the image gives the impression of being in three dimensions. Fluorescent microscopy utilizes a fluorescent dye to stain a cellular component. Immunofluorescence is a technique in which the dye is conjugated to an antigen or antibody so as to detect specific antibodies to the antigen or specific antigens to the antibody, respectively.

Electron microscopy substitutes the wavelengths of electrons for those of light waves. The shortness of the electron wavelengths provides the electron microscope with a resolving power of up to 0.005 μm. Two types of electron microscopy are available: transmission electron microscopy (TEM) and scanning electron microscopy (SEM). For TEM specimens must be dried and sectioned, which kills cells, but high magnifications (up to 200,000×) are possible. SEM is more suitable for obtaining three-dimensional images of specimens that do not have to be sectioned. SEM magnifications approach 10,000×.

Various staining techniques are available for the examination of bacteria by bright-field microscopy. Simple staining techniques require but one stain, while differential stains use two stains that enable the observer to differentiate certain groups of bacteria. The two most important differential stains are the Gram stain and the acid-fast stain. Other stains are available for detecting specific cellular structures such as capsules, flagella, and spores.

QUESTIONS FOR STUDY

1. What is meant by resolving power? How does wavelength affect it?
2. What is the advantage of the compound bright-field microscope over the electron microscope? Disadvantages?
3. What is the maximum magnification with the bright-field microscope? Electron microscope?

4. What is the purpose of using dark-field microscopy? Phase-contrast?
5. Describe the fluorescent antibody technique and how it is used.
6. What are some of the theories concerning the molecular mechanism of the Gram stain?
7. What advantages does the scanning electron microscope have over the transmission electron microscope?
8. What is the electrical charge on the surface of the bacterial cell? What is responsible for the charge?
9. What type of microscope would you use to identify the following: capsule, flagella, nuclear bodies, arrangement of the cell wall, viruses?
10. What groups of bacteria can be identified by the acid-fast stain?

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4. CHARACTERISTICS OF THE MICROBIAL WORLD: PROKARYOTES VS. EUKARYOTES

OBJECTIVES

To list the characteristics that distinguish prokaryotes from eukaryotes

To differentiate the terms genus, species, and strain

To briefly describe the difference(s) between the artificial and natural methods for classifying microorganisms

To list the basic components of a prokaryotic (bacterial) cell and their chemical composition

To list the basic components of a eukaryotic cell and their chemical composition

To describe the functions of the cytoplasmic membrane

To describe the chemical similarities and differences in the cell wall of gram-positive and gram-negative bacteria

To describe the functions of the capsule or slime layer of bacteria

To give the function of the following eukaryotic structures: endoplasmic reticulum, mitochondrion, Golgi-apparatus

To define: taxonomy, archaebacteria, hybridization, coccus, bacillus, pleomorphism, mesosome, peptidoglycan, periplasm, porin, endotoxin, osmosis, protoplast, spheroplast, capsule, slime layer, flagellum, chemotactic, pili, nucleoid, ribosome, endospore, vegetative cell, lysosome, pseudopodia

Classification
Prokaryotes, Eukaryotes, and Archaebacteria
Naming and Classification of Prokaryotes
Taxonomic Methods
Artificial Approach
Genetic analysis
Natural Classification
DNA-DNA Hybridization
DNA-rRNA Hybridization
Sequencing of 16S RNA
Comparative Analysis of Homologous Proteins
Classification and the Laboratory Technologist
Characteristics of the Bacteria
Size and Morphology
Pleomorphism
Cytoplasmic Membrane
Cell Wall
Gram-positive Bacteria
Gram-negative Bacteria
Osmosis and Cells Without Cell Walls
Extracellular Polymeric Substances
Flagella and Others Structures Associated with Movement
Pili (Fimbriae)
Structures Within the Cytoplasm
Nucleoids
Ribosomes
Inclusion Bodies
Endospores
Sporulation Process
Germination
Characteristics of Eukaryotes
Cytoplasmic Membrane
Cell Wall
Endoplasmic Reticulum
Golgi
Mitochondria
Flagella, Cilia, and Cytoplasmic Streaming
Nucleus

Each day new microbial properties are revealed by our advances in microscopy, biochemistry, and molecular biology. These advances not only give us greater insight into microbial life and the factors that affect it, but they also give us insight into the properties of higher forms of life. The results obtained from the study of metabolic, physiological, and genetic characteristics in microbial models have provided us with a better understanding of many mammalian functions.

The chemical composition and molecular architecture of the various components of the microbial cell have special significance to medical and allied health personnel. The inhibition of growth or destruction of disease-producing as well as non-disease-producing microorganisms is related to the presence or absence of various microbial structures and to their biochemical composition.

The purposes of this chapter are to demonstrate how the various characteristics of microorganisms are used to develop a classification scheme and to describe the structure and function of microbial components and their importance, if any, in the infectious disease process.

CLASSIFICATION

Once microorganisms had been discovered and observed to be of various sizes, shapes, and metabolic potential, scientists sought to place them in some type of classification scheme. The science of classification is called *taxonomy*. Before the twentieth century the biological world was divided into two major kingdoms: animals and plants. The classification of plants and animals is based on phylogenetic relationships, that is, common ancestry. Such an evolutionary relatedness can be determined from the examination of fossil records, which have been readily available for plants and animals but, with one or two exceptions, have not

Table 4-1. Important Characteristics Distinguishing Prokaryotes from Eukaryotes

Characteristic or Structure	Eukaryote	Prokaryote
Size (avg)	5–10 μm	1–3 μm
Cell wall	Present in fungi and algae	Present in all bacteria except *Mycoplasma*
Cytoplasmic membrane	Unit membrane has sterols	Unit membrane, no sterols except in *Mycoplasma*
Nuclear membrane	Present	Absent
Nucleolus	Present	Absent
DNA	More than one chromosome in nucleus; chromosomes are associated with proteins called histones	Single chromosome; no associated proteins
Ribosomes	Sedimentation coefficient of 80S	Sedimentation coefficient of 70S
Respiration	Associated with mitochondrion	Associated with cytoplasmic membrane
Reproduction	Sexual and asexual	Asexual (binary fission)
Cytoplasmic streaming	Present	Absent

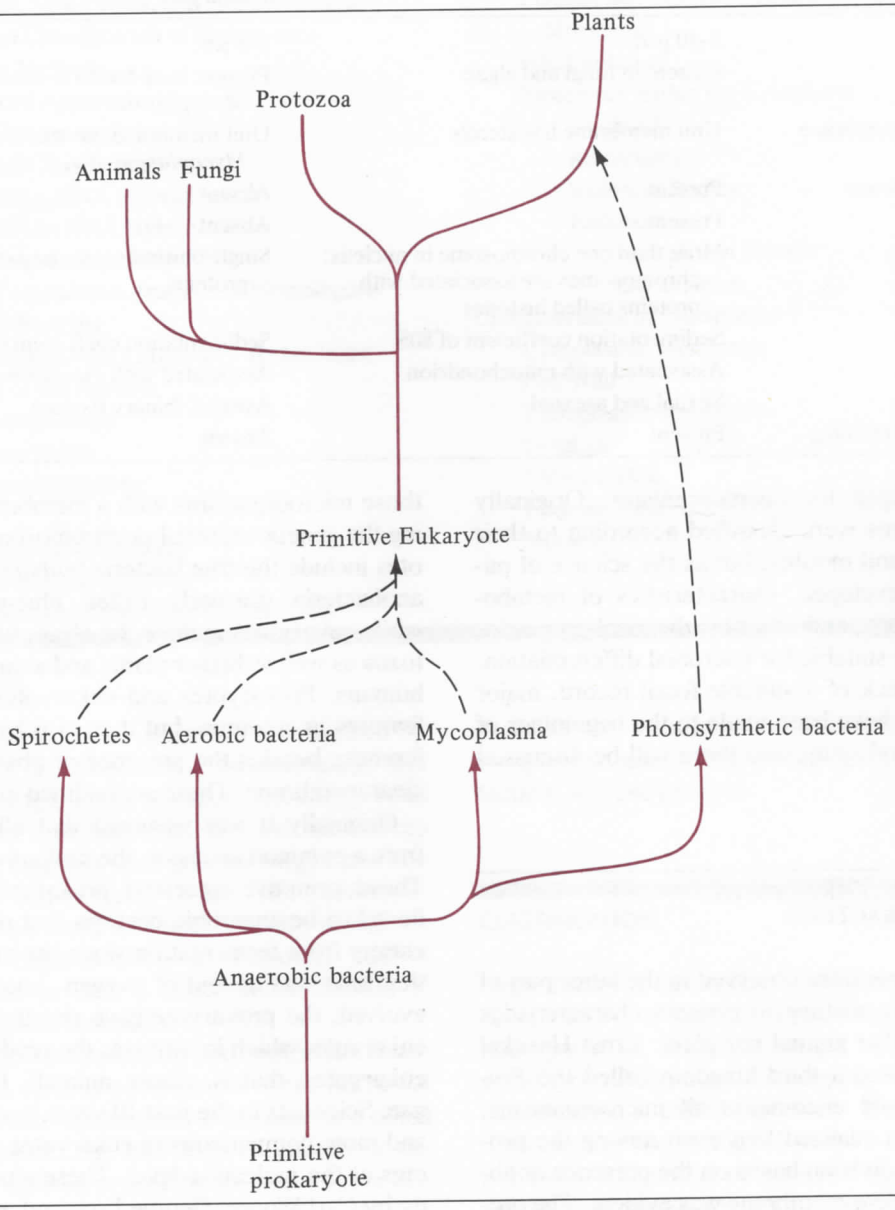
been discovered for microorganisms. Originally microorganisms were classified according to their morphology and motility, but as the science of microbiology developed, characteristics of metabolism, physiology, and other biochemical properties became more suitable for microbial differentiation. Despite the lack of a suitable fossil record, major contributions have been made to the beginnings of a bacterial phylogeny, and these will be discussed later.

PROKARYOTES, EUKARYOTES, AND ARCHAEBACTERIA

Microorganisms were observed in the latter part of the nineteenth century to possess characteristics that were neither animal nor plant. Ernst Haeckel in 1866 proposed a third kingdom called the *Protista* that would encompass all microorganisms. Scientists later realized that even among the protists a natural division based on the presence or absence of a nuclear membrane was evident. The protists were, therefore, divided into two groups, *prokaryotes* (from the Greek *protos*, primitive, and *karyon*, nucleus) or those microorganisms without a membrane surrounding the genetic material; and *eukaryotes* (Gr. *eu*, true, and *karyon*, nucleus) or those microorganisms with a membrane surrounding the genetic material or chromosomes. Prokaryotes include the true bacteria (*eubacteria*) and Cyanobacteria (formerly called blue-green algae), while eukaryotes include the algae, fungi, and protozoa as well as higher plants and animals including humans. Prokaryotes and eukaryotes have many features in common, but they also have many differences besides the presence or absence of a nuclear membrane. These are outlined in Table 4-1.

Originally it was assumed that all life derived from a common ancestor, the prokaryote (Fig. 4-1). These primitive ancestral prokaryotes were believed to be anaerobic bacteria that obtained their energy from fermentation since the atmosphere at that time was devoid of oxygen. Later, as oxygen evolved, the prokaryote gave rise to the primitive eukaryote, which in turn was the predecessor of all eukaryotes, that is, plants, animals, fungi, and algae. Scientists in the past 10 years have made more and more comparisons of eukaryotes and prokaryotes at the molecular level. These studies, primarily by Carl Woese, George Fox, and associates, indicate that living forms fall into one of three categories: prokaryotes, eukaryotes, and *archaebacteria* (Fig. 4-2). These three groups possess similar organizational properties but differ at the molecular level. The archaebacteria resemble true

Fig. 4-1. Phylogenetic tree based on conventional taxonomic schemes that assume all life was derived from a common ancestor.



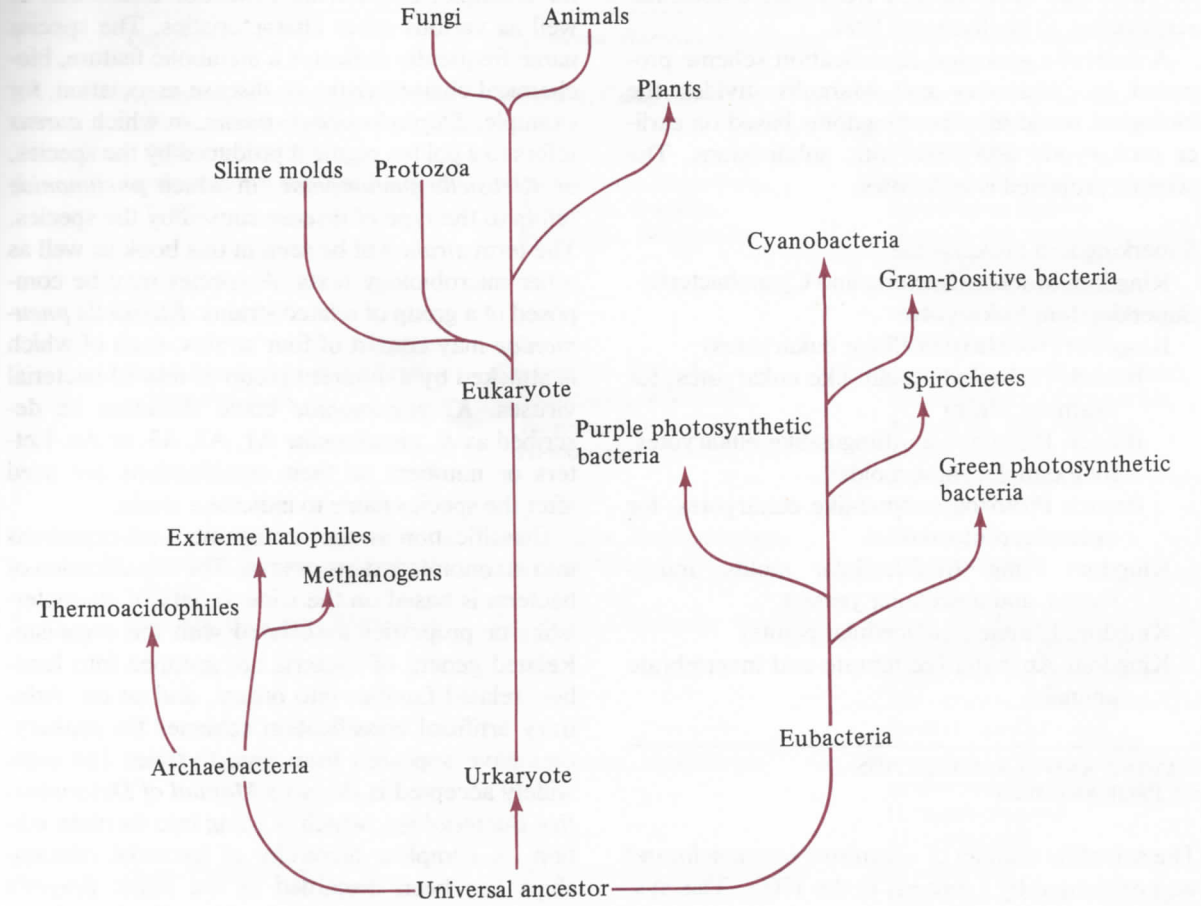


Fig. 4-2. Phylogenetic scheme based on 16S rRNA sequence analysis. (Adapted from G. E. Fox et al., *Science* 209:457, 1980. Copyright 1980 by the American Association for the Advancement of Science.)

bacteria (eubacteria) in the usual organizational ways, that is, size, absence of organelles such as chloroplasts and mitochondria, absence of a nuclear membrane, and ribosome size, but in some respects they are no more closely related to prokaryotes than are prokaryotes to eukaryotes. What is it that makes the archaebacteria unique? First, the members of the archaebacteria are found in harsh environments that exclude other organisms. These environments include (1) areas of very high salt concentration (extreme halophilic bacteria), (2) hot acid environments (thermoacidophilic bacteria), and (3) anaerobic environments where methane is produced from carbon dioxide and hydrogen gas (methanogenic bacteria). Three of the several molecular traits distinguishing the archaebacteria from other bacteria are as follows: (1) Archaebacteria have cell walls that are devoid of muramic acid, a component found in all bacteria that have cell walls. (2) Archaebacteria possess membrane lipids in which the fatty acid chains are ether-linked and branched, instead of the ester-linked and straight-chained lipids found in the true bacteria. (3) Archaebacteria show a sensitivity to certain an-

tibiotics that is directly the opposite to that for true bacteria. The evidence that the archaebacteria are phylogenetically distinct from prokaryotes and eukaryotes has been derived from macromolecular sequencing, to be discussed later.

A currently accepted classification scheme proposed by Whittaker and Margulis divides the biological world into five kingdoms based on earlier prokaryotic and eukaryotic subdivisions. The scheme proposed is as follows:

Superkingdom Prokaryotae
- Kingdom Monera (Bacteria and Cyanobacteria)

Superkingdom Eukaryotae
- Kingdom Protista (unicellular eukaryotes)
  - Branch Protophyta (plant-like eukaryotes, for example, algae)
  - Branch Protomycota (fungus-like eukaryotes, for example, slime molds)
  - Branch Protozoa (animal-like eukaryotes, for example, protozoa)
- Kingdom Fungi (multicellular molds, mushrooms, and unicellular yeasts)
- Kingdom Plantae (multicellular plants)
- Kingdom Animalia (vertebrate and invertebrate animals)

NAMING AND CLASSIFICATION OF PROKARYOTES

The scientific naming of organisms (*nomenclature*) was originated by Linnaeus in the 1700s. This system is based on a classification unit that incorporates two names (binomial nomenclature) called *genus* and *species*. The bacterial species, which is the basic classification unit, signifies a distinct member of a group of microorganisms that possess similar characteristics but differ in important ways from related groups of bacteria in other independent characteristics. The genus (plural *genera*) is capitalized, while the species name is not. Both words are italicized, for example, *Pseudomonas aeruginosa*. Much can be learned from the genus and species names. The genus name of bacteria often indicates the morphology of the species, for example, *Streptococcus* (chain of spherical cells), *Bacillus* (rod-shaped), *Spirochaeta* (spiral-shaped), but it can also indicate the organism's discoverer, for example, *Escherichia* (Theodor Escherich) as well as various other characteristics. The species name frequently indicates a metabolic feature, biochemical characteristic, or disease association, for example, *Staphylococcus aureus*, in which *aureus* refers to a golden pigment produced by the species, or *Klebsiella pneumoniae*, in which *pneumoniae* refers to the type of disease caused by the species. The term *strain* will be seen in this book as well as other microbiology texts. A species may be composed of a group of related strains. *Klebsiella pneumoniae* may consist of four strains, each of which is attacked by a different group of related bacterial viruses. *K. pneumoniae* could therefore be described as *K. pneumoniae* A1, A2, A3, or A4. Letters or numbers or their combinations are used after the species name to indicate a strain.

Classification is the arrangement of organisms into taxonomic groups or taxa. The classification of bacteria is based on the wide variety of characteristics or properties associated with the organism. Related genera of bacteria are grouped into families, related families into orders, and so on. Arbitrary artificial classification schemes for prokaryotes have appeared from time to time. The most widely accepted is *Bergey's Manual of Determinative Bacteriology*, which is going into its ninth edition. A complete hierarchy of bacterial relationships has been discarded in the latest *Bergey's Manual*, which is restricted to identification of bacteria. The current edition has classified prokaryotes into the Kingdom Procaryotae, under which there are various sections. Each section represents a specific group of bacteria that has been separated based on one or more features such as staining characteristics, morphology, oxygen requirements, physiological responses, spore formation, and other characteristics. Each section, which may be further subdivided into families and orders, contains a number of genera. A breakdown of the various sections and some of their characteristics appears in Table 4-2.

Table 4-2. Outline of those taxonomic divisions found in *Bergey's Manual of Determinative Bacteriology* that contain the major human pathogens

Section	Section description	Important genera	Diseases
1	The Spirochetes	*Treponema, Borrelia, Leptospira*	Syphilis (*Treponema*), undulant fever (*Borrelia*)
2	Aerobic/Microaerophilic, Motile, Helical/Vibroid, Gram-negative Bacteria	*Spirillum, Campylobacter*	Rat-bite fever (*Spirillum*), intestinal disease (*Campylobacter*)
4	Gram-negative Aerobic Rods and Cocci	*Pseudomonas, Legionella, Neisseria, Brucella, Bordetella, Francisella,*	Opportunistic* (*Pseudomonas*), legionnaires disease (*Legionella*), meningitis and gonorrhea (*Neisseria*), brucellosis (*Brucella*), whooping cough (*Bordetella*), tularemia (*Francisella*)
5	Facultatively Anaerobic Gram-Negative Rods	*Escherichia, Shigella, Salmonella, Klebsiella, Yersinia, Proteus, Vibrio, Hemophilus*	Intestinal disease (*Escherichia*), opportunistic* (*Escherichia, Klebsiella*, and *Proteus*), plague (*Yersinia*), cholera (*Vibrio*), meningitis (*Hemophilus*)
6	Anaerobic Gram-Negative, Straight, Curved and Helical Rods	*Bacteroides, Fusobacterium, Leptotrichia*	Infections of oral cavity, opportunistic*
9	The Rickettsia and Chlamydias	*Rickettsia, Chlamydia, Coxiella*	Typhus and Rocky Mountain spotted fever (*Rickettsia*), psittacosis and genitourinary tract disease (*Chlamydia*), Q fever (*Coxiella*)
10	The Mycoplasma	*Mycoplasma, Ureaplasma*	Pneumonia (*Mycoplasma*), genitourinary tract disease (*Ureaplasma* and *Mycoplasma*)
12	Gram-Positive Cocci	*Staphylococcus, Streptococcus*	Skin infections (*Staphylococcus*), rheumatic fever (*Streptococcus*), opportunistic* (*Staphylococcus*)
13	Endospore-Forming Gram-Positive Rods and Cocci	*Bacillus, Clostridium*	Anthrax (*Bacillus*); tetanus, botulism, and gas gangrene (*Clostridium*)
15	Irregular, Nonsporing, Gram-Positive Rods	*Corynebacterium, Actinomyces*	Diphtheria (*Corynebacterium*), lesions of face and lungs (*Actinomyces*)
16	Mycobacteria	*Mycobacterium*	Tuberculosis and leprosy
17	Nocardioforms	*Nocardia*	Lesions of skin

*Opportunistic pathogens are organisms that cause disease in individuals compromised by some underlying illness or condition. Most opportunistic infections occur in the hospital.

Source: From J. G. Holt (Ed.), *Bergey's Manual of Determinative Bacteriology* (9th ed.). Baltimore: Williams & Wilkins, 1984.

TAXONOMIC METHODS

The arrangement of organisms into various taxonomic groups is based on their degree of relatedness. The morphological, biochemical, and physiological characteristics as well as staining properties that are used to differentiate bacteria represent an artificial scheme. More recent advances in genetics and biochemistry have made classification by the natural phylogenetic relationship possible. The methods used to classify bacteria via phylogenetic relatedness are based on sequence analysis of the macromolecules DNA, RNA, and protein.

Artificial Approach

Much of bacterial taxonomy revolves about the comparison of various microbial traits such as morphology and physiological characteristics. Morphological traits are usually stable characteristics that are not influenced by environmental factors. Physiological traits, however, are subject to some variation. The temperature and pH requirements for growth or the fermentation products released by bacteria may be influenced by the environment. The kind of trait that can be used to separate or classify certain groups of bacteria may vary from one group to another. Chemical composition of cell constituents is a technique that can be used when physiological and morphological characteristics are unsatisfactory. The qualitative analysis of cell walls or lipids can be used to discriminate between certain groups of bacteria. Lipid composition, for example, was one characteristic used to differentiate archaebacteria from eubacteria. By evaluating various morphological or nonmorphological characteristics, bacteria can be arranged into various taxonomic groups. Unfortunately, not all scientists agree on which microbial characteristics are most important. Some microbial properties are given more weight than others by one investigator, while another may suggest that these properties should not be considered for classification. A technique called *numerical taxonomy* gives equal weight to all characteristics examined. All of the characteristics of the organisms are evaluated by a computer. A percentage of the total number of traits held in common between the organisms is expressed as a similarity profile. Those organisms showing similar profiles are placed into specific groups or taxa.

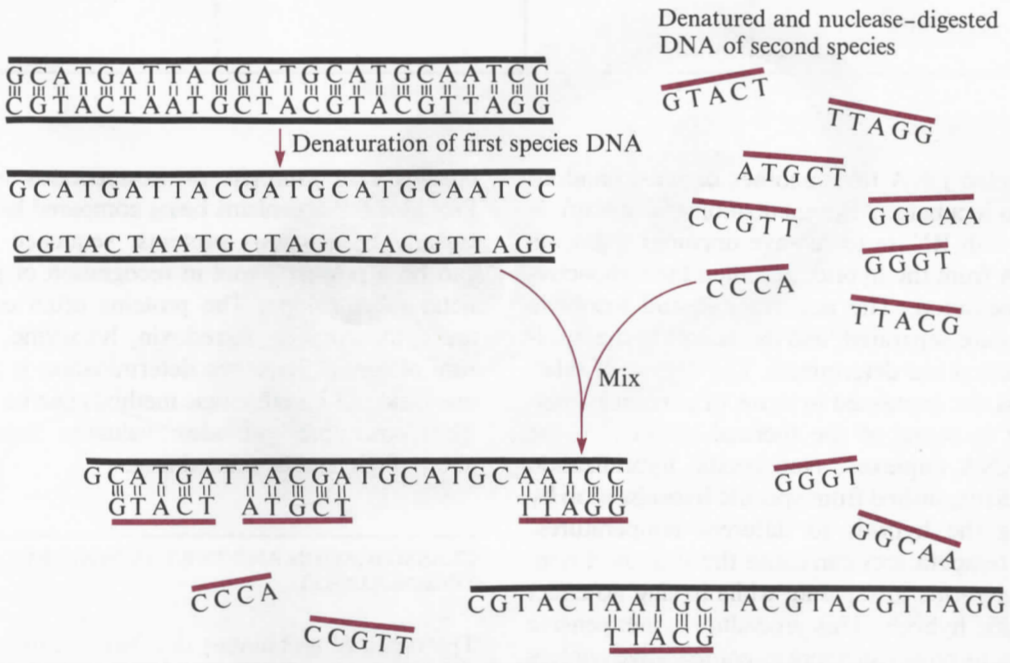
GENETIC ANALYSIS. Base composition is a constant characteristic of organisms. The relatedness of some microorganisms can be determined by evaluating the DNA base composition, that is the G/C content of the organisms. Guanine pairs with cytosine and adenine with thymine in the DNA molecule, but the total number of G/C base pairs to A/T base pairs varies from one species to another. DNA base composition is considered one of the required characteristics for a description of a new species. It is an exclusionary determinant in that organisms whose DNA base composition differs by more than 10 mol percent should not be considered members of the same genus. Two microorganisms that have the same G/C content could still be the same or different since the sequence of bases in each of them could be totally different. In classifying bacteria DNA base composition is used primarily in combination with other chemotaxonomic markers to resolve taxonomic problems and in supporting phylogenetic data. The G/C content, which varies from 23 percent to 75 percent in microorganisms, can be determined using the following formula:

$$\text{Percent G + C} = \frac{\text{G + C}}{\text{A+T+G+C}} \times 100\%$$

Natural Classification

Classification of bacteria based on natural relationships is called *phylogenetic* classification. Without a meaningful fossil record bacterial taxonomists must rely on techniques for determining the sequences of the informational molecules DNA, RNA, and protein. The differences in the sequences of bases or amino acids among informational molecules from different microorganisms is an indication of evolutionary relatedness. This assumption is based on studies of amino acid changes of functionally similar proteins in higher animals. These eukaryotic studies have indicated that the extent of change in the sequences of the informa-

Fig. 4-3. DNA-DNA hybridization technique for determining the degree of relatedness between various strains. The DNA from one species is denatured to produce single strands and is then trapped on some type of matrix. The DNA of the second species is made radioactive and then denatured and digested with a nuclease to produce short pieces. The DNA's are mixed, heated, and then cooled slowly to allow any complementary stretches of bases to form double-stranded DNA. The relative amount of double-stranded DNA indicates the degree of relatedness between the two species.



tional molecules is roughly proportional to the time that has elapsed since their divergence and the formation of new species. The methods for classifying prokaryotes on the basis of their phylogenetic relatedness follow:

DNA-DNA HYBRIDIZATION. DNA from different sources can be melted to produce single strands. After melting the single-stranded DNA from the different sources is mixed and the reassociation analyzed. The degree of reassociation (hybridization) is referred to as *percent homology* (Fig. 4-3). The technique is relatively easy to perform and inexpensive and gives an average measure of the similarity of DNA base sequence over the entire genome. This technique can be used only for closely related organisms since representatives of a genus that appear to be closely related by other phylogenetic studies may share little DNA homology. DNA-DNA hybridization is very useful in detecting misclassified strains and for assigning newly described organisms to existing taxa.

DNA-rRNA HYBRIDIZATION. The ribosome, which is involved in protein synthesis, is the only cellular organelle present in all living organisms. It is composed of protein and rRNA, which have been shown to possess highly stable sequences. Ribosomal RNA's are direct transcripts from portions of the cell's DNA, and therefore some homology exists between the two molecules. The hybridization procedure requires the labeling of rRNA with radioactive material. Labeled RNA (23S or 16S or both) from one organism is mixed with excess sin-

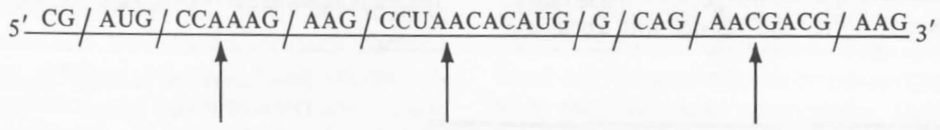


Fig. 4-4. 16S RNA analysis. A portion of 16S RNA is subjected to digestion by ribonuclease. The fragments released are from one to several nucleotides in length. Each fragment ends in *G*. Only fragments with six or more nucleotides (*arrows*) are placed in a nucleotide-word dictionary for comparison with fragments of 16S RNA from other species.

gle-stranded DNA from another organism and allowed to incubate. After incubation the mixture is treated with RNase to remove unpaired segments of rRNA from the hybrid, and then the radioactivity is measured. The acid soluble and insoluble fractions are separated, and the counts in the insoluble fraction are determined. The degree of relatedness is not expressed in terms of percent homology but in terms of the thermal stability of the RNA-rRNA duplexes. Nonspecific hybridization can be distinguished from specific hybridization by exposing the hybrids to different temperatures. Certain temperatures can cause the strands of nonspecific hybrids to separate while having no effect on specific hybrids. This procedure is inexpensive but is not as powerful a tool as comparative nucleotide sequencing of the 16S rRNA molecule.

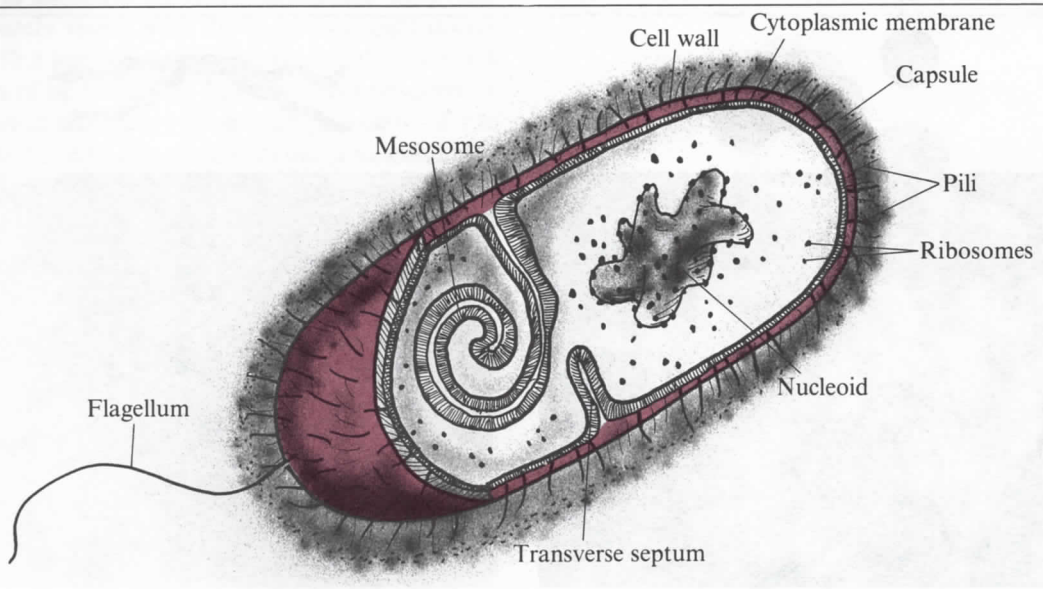
SEQUENCING OF 16S rRNA. Sequencing of 16S rRNA was the technique used by Woese and Fox for the separation of microorganisms into three kingdoms, eubacteria, archaebacteria, and eukaryotes. In this procedure 16S rRNA from various microbial species is digested with an enzyme. The fragments that are produced vary in length from 1 to 20 nucleotides. The base sequence of each fragment is determined, and fragments of six nucleotides or more are compiled into a "dictionary." The dictionaries of two organisms can then be compared to determine the degree of association between them (Fig. 4-4). These experiments have demonstrated that one part of the 16S rRNA has remained relatively stable during evolution while the other part has shown considerable variability.

COMPARATIVE ANALYSIS OF HOMOLOGOUS PROTEINS. Provided the organisms being compared have functionally homologous proteins, sequence analysis can be a powerful tool in recognition of phylogenetic relationships. The proteins often examined are cytochrome c, ferredoxin, lysozyme, and serum albumin. Sequence determination is a laborious task, but immunologic methods can be utilized. The procedure provides valuable information about phylogenetic relatedness.

CLASSIFICATION AND THE LABORATORY TECHNOLOGIST

The types of techniques that have been described for the classification of bacteria are for the most part unsuitable for identification in the clinical laboratory. Rapid test systems are more suitable in routine laboratory studies. These tests include fermentation properties, oxidase test, coagulase tests, or various serological determinations. In the clinical laboratory standardized schemes for the identification of organisms from clinical samples are available, but it is important to remember that these schemes are not suitable for the differentiation of pathogenic and nonpathogenic species from their natural habitats.

Fig. 4-5. A. Basic morphology of a bacterial cell. Some of the features, for example, flagella and extracellular polymeric substances (capsule), may not be present in all bacterial cells. B. Electron micrograph of a rod-shaped bacterium illustrating basic morphology. (*C* = capsule; *TS* = transverse septum; *CW* = cell wall; *M* = mesosome; *CM* = cytoplasmic membrane; *n* = nuclear area; *R* = ribosome.) (From D. J. Ellar, D. G. Lundgren, and R. A. Slepecky, *J. Bacteriol.* 94:1189, 1967.)



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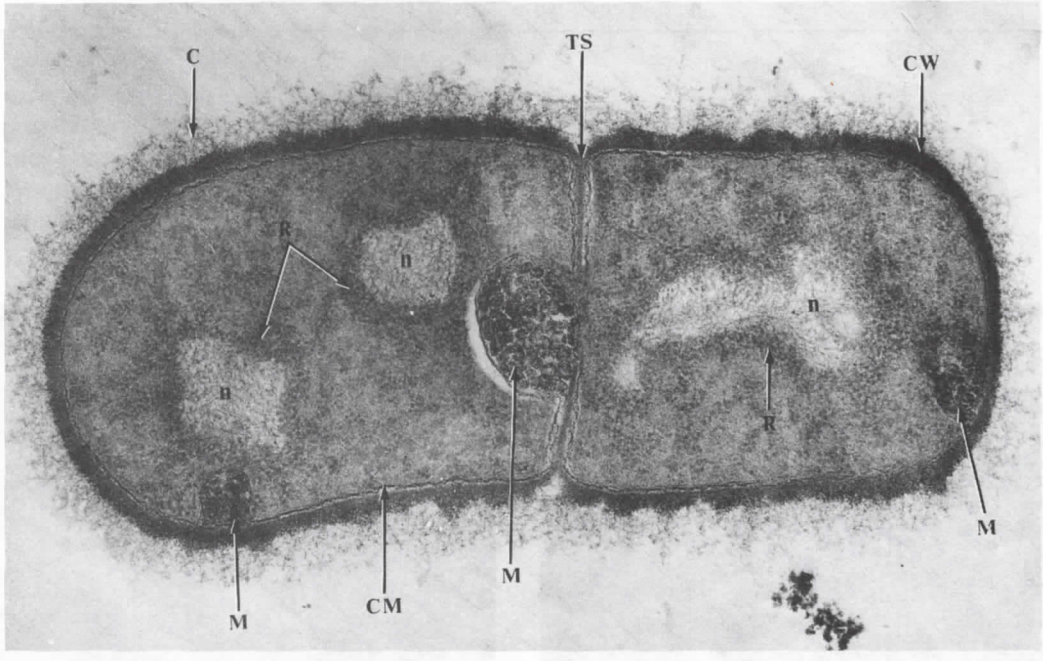

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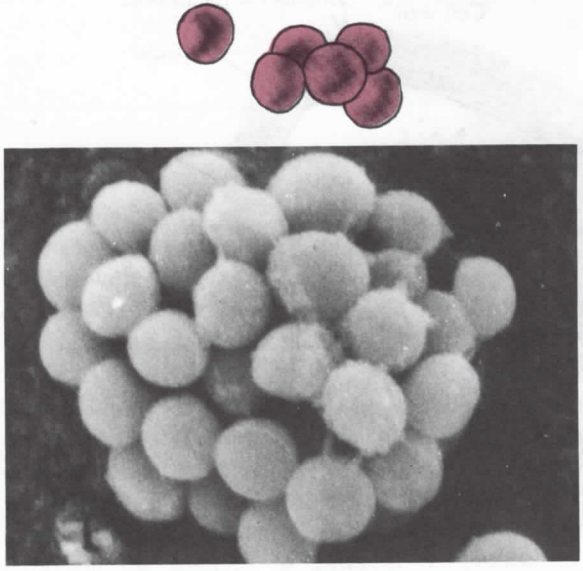
Fig. 4-6. Shapes of bacteria. A. Spherical (coccus). Scanning electron micrograph of staphylococci (×14,000). (Courtesy Z. Yoshii.) B. Rod (bacillus). Scanning electron micrograph of bacilli. (From T. J. Kerr, et al., *Appl. Environ. Microbiol.* 46:1201, 1983. C. Spiral. Micrograph of rumen treponemes. (From A. Ziolecki, *Appl. Environ. Microbiol.* 37:131, 1979.) D. Square. Micrograph is phase-contrast (×1600 before 20% reduction). (From D. Stoeckenius, *J. Bacteriol.* 148:352, 1981.)



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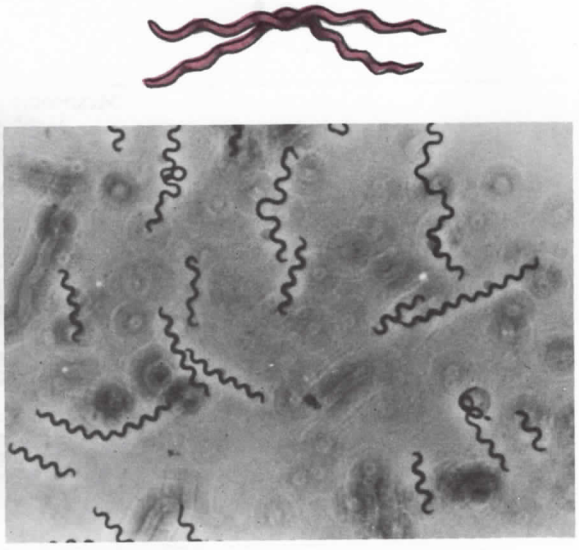

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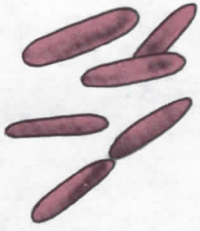

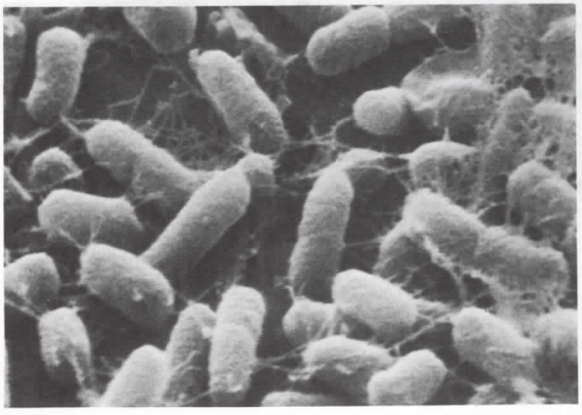

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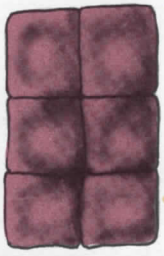
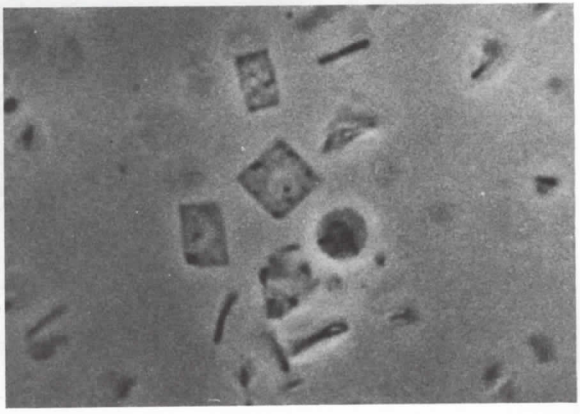




D

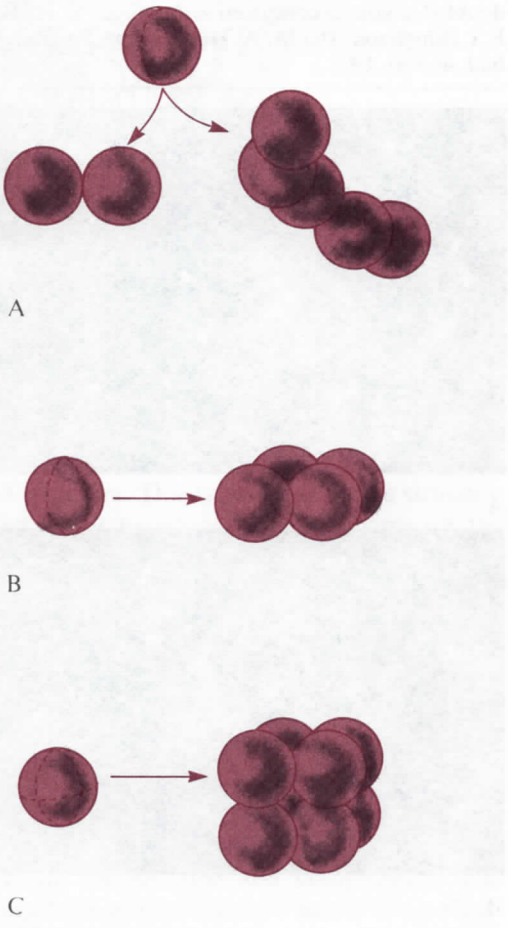
CHARACTERISTICS OF THE BACTERIA

SIZE AND MORPHOLOGY

Bacteria show considerable variation in size, from cells barely visible with the compound light microscope (0.2 μm) to spiral organisms that may reach lengths of up to 60 μm. Most bacteria observed in the clinical laboratory have average lengths of 1 to 3 μm and can be observed with the compound light microscope. Some of the basic structures that can be observed microscopically in a typical bacterial cell are illustrated in Figure 4-5.

Bacteria have four basic shapes: spherical, rod, spiral, and square (Fig. 4-6). Spherical cells are called *cocci* (singular *coccus*) and may exist in different arrangements. Cocci that divide usually remain attached to one another, and the arrangement that follows is based on the plane of division (Fig. 4-7). Cocci that divide along the same axis may form chains of cocci varying in length from 2 to 20 cocci. A pair of cocci are called *diplococci*, while chains of 4 to 20 are called *streptococci*. Cocci that divide in two planes form a tetrad. Division in three planes results in the formation of a group of eight called *sarcina* or large clusters of cocci called *staphylococci*. Rod-shaped cells are called *bacilli* (sing. *bacillus*). They divide in one plane and are sometimes observed to be in short chains, but usually they appear singly (see Fig. 4-6). The term *bacillus*, which signifies a type of morphology, should not be confused with the taxonomic genus called *Bacillus*. Spiral-shaped micoorganisms, which appear in a helical or corkscrew shape, divide in one plane and separate at cell division. The square-shaped bacteria are a recently discovered group found in those areas having a high salt content (Dead Sea, Great Salt Lake). Square bacteria are not known to be involved in any disease process and therefore will not be discussed further in this text.

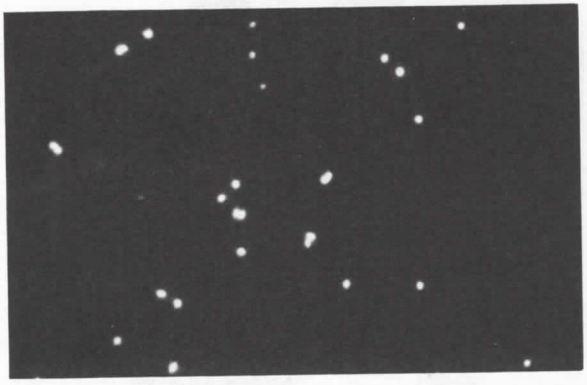
Fig. 4-7. Arrangements of cocci based on the plane of cell division. A. Division in one plane produces diplococci or streptococci. B. Division in two planes produces a tetrad. C. Division in three planes produces a group of eight (sarcina) or clusters (staphylococci).



Pleomorphism

Microorganisms do not always maintain the same shape. Changes in chemical or physical factors can influence the size and morphology of bacteria. Changes in the nutrient content of the media, the presence of antibiotics that affect cell wall synthesis, or an increase in hydrostatic pressure are some of the factors that can influence the shape of organisms. Cocci may be smaller because of these influences, but the change is so slight that it can hardly be detected microscopically. The most recognizable changes are those that involve rod-shaped microorganisms. Rod-shaped microorganisms grown

Fig. 4-8. Effect of nutrition on the size of rod-shaped bacteria. Nutrient-starved *Vibrio cholerae* cells were stained with a fluorescent dye and observed by epifluorescence microscopy. A. Cells before nutrient addition. B. Cells 2 hours after nutrient addition. C. Cells 24 hours after nutrient addition. Note that the cells have doubled in size as compared to A. (From R. M. Baker, F. L. Singleton, and M. A. Hood, *Appl. Environ. Microbiol.* 46:930, 1983.)



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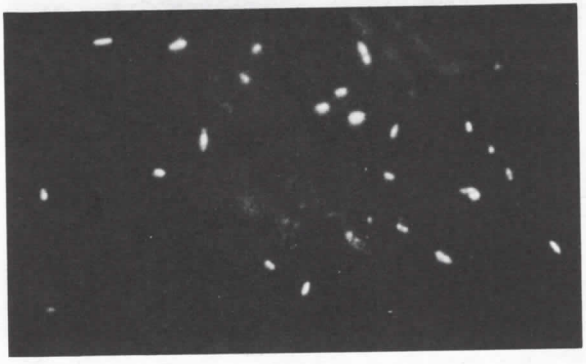

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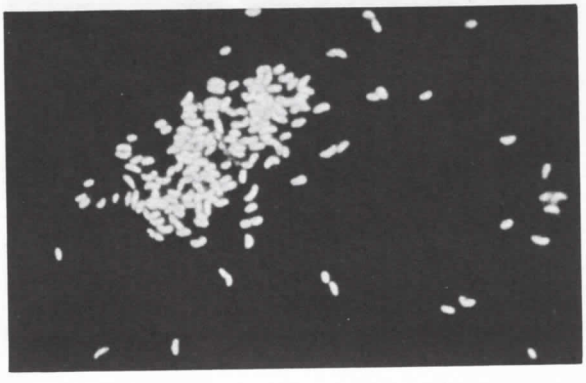

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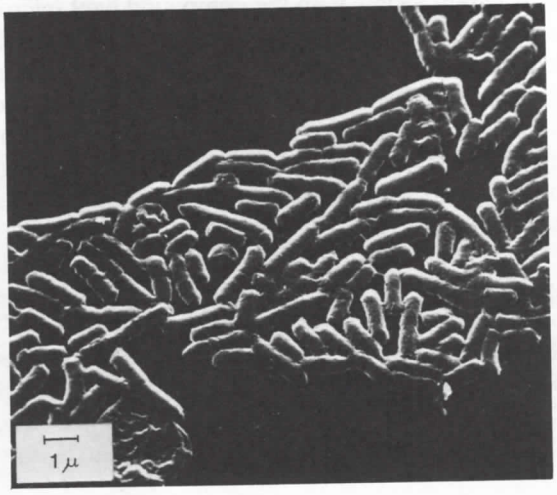
Fig. 4-9. Effect of subinhibitory concentrations of penicillin on rod-shaped bacteria. A. Untreated control. B. Cells after treatment with ampicillin. Note the filamentous nature of the cells. (From O. Zak and F. Kradolfer, *Rev. Infect. Dis.* 1:862, 1979.)



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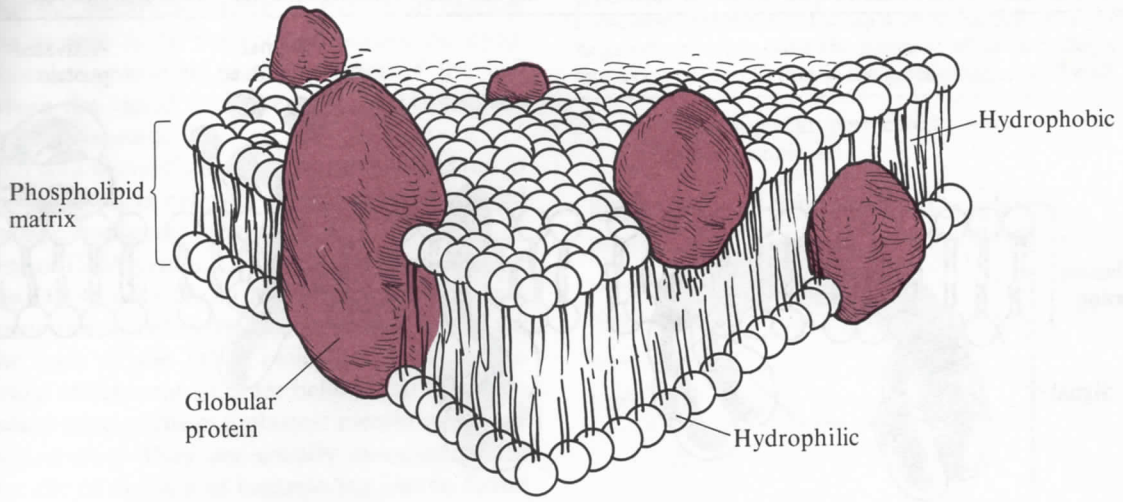


Fig. 4-10. Cytoplasmic membrane model. Globular proteins are embedded in a phospholipid matrix. (Adapted from S. J. Singer and G. I. Nicolson, *Science* 175:720, 1972.)

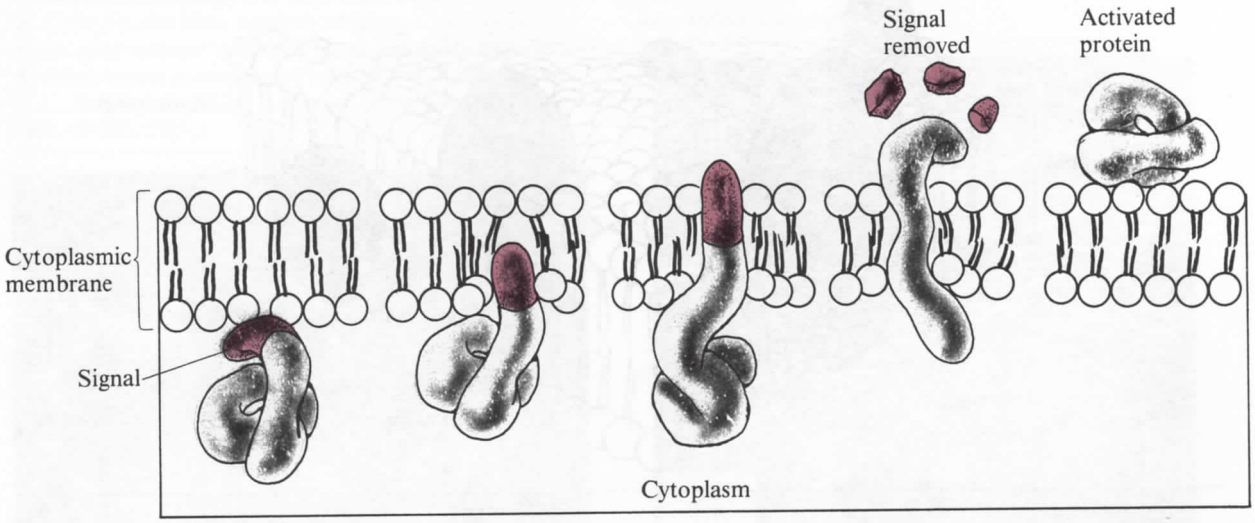
in a medium supporting maximum growth may be two to four times the length of the same organisms grown in a nutritionally poor medium (Fig. 4-8). Rod-shaped microorganisms in the presence of penicillin or related antibiotics that affect cell wall synthesis often produce filaments (Fig. 4-9). Pleomorphism in bacteria prevents the taxonomist from using size and morphology as a means of classifying bacteria.

CYTOPLASMIC MEMBRANE

The cytoplasm of the cell is surrounded by a membrane referred to as the *cytoplasmic membrane, cell membrane*, or *plasma membrane*. The cell membrane is a dynamic flexible structure that is actively and passively engaged in the transport of many different molecules, both organic (fatty acids, carbohydrates, proteins) and inorganic (water, potassium, magnesium). These transport properties are associated with the structure of the membrane as well as its electrical charge.

The cytoplasmic membrane when observed by electron microscopy appears to consist of a three-layered structure. This typical membrane structure is called a *unit membrane* (another name for the phospholipid bilayer characteristic of the cytoplasmic membrane), which distinguishes it from more complex membrane systems. Chemical characterization of the membrane indicates that it consists of phospholipids and proteins. Singer and Nicolsen in 1972 proposed a model called the *fluid mosaic model* to explain the structure of the membrane based on biochemical and biophysical data. This model (Fig. 4-10) shows that there are two phospholipid layers within which are embedded proteins of various size and composition. The water insoluble groups (the fatty acids) of the phospholipids are oriented in such a way that they associate with each other and are not exposed to the environment. The hydrophilic groups (PO_4, for example) of the phospholipid, are in direct contact with the environment (Fig. 4-10). The proteins of the cytoplasmic membrane may bridge both phospholipid layers, or they may be associated with one layer or the other. The more ionic amino acids of the protein face the surface while the nonionic amino acids are oriented toward the interior or hydrophobic portion of the phospholipid bilayer.

Fig. 4-11. Signal hypothesis for the transport of secretory proteins across the cytoplasmic membrane. Each protein possesses a terminal sequence of amino acids called the signal that enables the protein to be transported across the cytoplasmic membrane. Once the signal has passed through the membrane it is cleaved.



The cytoplasmic membrane performs several functions in the bacterial cell:

1. Semipermeable barrier. The charge on the surface of the membrane can influence the type of molecule that enters the cytoplasm. Most substances, even the smallest ions, do not passively enter the cell, either because of the electrical charge on the membrane surface or because of the lack of suitably sized pores in the membrane. Most substances enter the cytoplasm of the cell only by selective transport mechanisms (see Chapter 7).
2. Active transport. The cell is capable of removing nutrients from the environment against a concentration gradient. Substances such as amino acids, sugars, and other small molecules that are ordinarily in low concentration in the cell's environment can be concentrated within the cell. These substances are carried across the cytoplasmic membrane by a process referred to as active transport. The active transport system utilizes special proteins in the cytoplasmic membrane called *permeases*. The mechanisms of active transport are discussed at more length in Chapter 7.
3. Site of respiratory activity. The ability of bacterial cells to produce much of their energy is associated with a variety of proteins and enzymes located in the cytoplasmic membrane. This type of activity in eukaryotic cells is associated with organelles called mitochondria.
4. Secretion. Many substances produced in the cytoplasm of the cell must be transferred to the exterior surface of the cell or to those structures that are external to the cytoplasmic membrane. Proteins that are required for cell wall synthesis, for example, are produced in the cytoplasm. Various toxins that are produced by microorganisms and may be involved in the disease process or inhibit other bacteria are also produced in the cytoplasm and must be transported to the exterior. In addition many bacteria produce enzymes that are secreted outside the cell to break down macromolecules into smaller units that can be carried across the cytoplasmic membrane into the cell cytoplasm. Recent experiments have demonstrated that when proteins are exported

from the cell they include an additional sequence of amino acids, called the *signal sequence*, located at one end of the molecule. It is believed that the electrical charge of the signal enables the protein to be transported across the cytoplasmic membrane to the exterior of the cell where the signal is cleaved (Fig. 4-11) and the protein released.

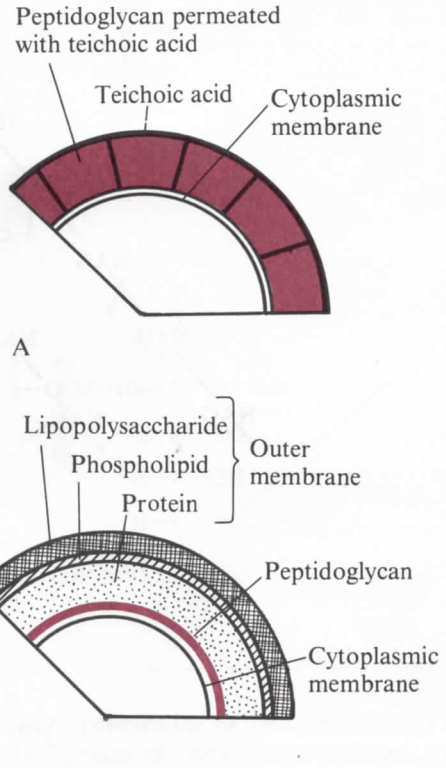
5. Cell wall biosynthesis. The enzymes involved in the assembly of cell wall components are located in the cytoplasmic membrane.
6. Site of DNA synthesis. During the replication of the DNA molecule in the cell the cytoplasmic membrane is believed to be an anchoring site for the ends of the DNA molecule. These membrane attachment sites are believed to be invaginated areas of the cytoplasmic membrane called *mesosomes*. They are usually associated with the site of division of bacteria but can be found elsewhere in the cell (see Fig. 4-5).

CELL WALL

The cell wall is composed of several polymers that provide strength to the cell and protect the internal cytoplasmic membrane and its contents from environmental factors. The relative rigidity of the cell wall is also responsible for the shape of the organism. Bacteria are normally found in environments that are hypotonic (low solute concentration), that is, water has a tendency to pass from the environment into the cytoplasm of the cell. The cell would burst if it were not for the strength of the cell wall and its special architecture.

The bacterial world with only a few exceptions is divided into two basic cell wall types, *gram-positive* and *gram-negative*, on the basis of a staining procedure called the Gram's stain (see Chap. 3). Division into gram-positive and gram-negative is predicated on marked differences in the physical and chemical makeup of the cell wall. A cell is gram-positive if it resists decolorization by alcohol after the application of a primary stain and gram-negative if it is decolorized by alcohol after primary staining. The backbone material of both cell types

Fig. 4-12. Relative composition and position of cell wall components. A. Gram-positive cell wall. The peptidoglycan is believed to be permeated by teichoic or teichuronic acid that reaches the surface of the cell. B. Gram-negative cell wall. Note the thinness of the peptidoglycan as compared to that in the gram-positive cell wall.



is the *peptidoglycan* layer (also called *murein, glycopeptide layer,* or *mucopeptide*).

Gram-positive Bacteria

The cell wall of gram-positive bacteria is 50 to 60 percent peptidoglycan, with the remainder being teichoic acids or teichuronic acids and polysaccharides (Fig. 4-12A). The peptidoglycan is composed of N-acetyl glucosamine and N-acetyl muramic acid bonded together by 1–4 linkages. The polysaccharide chains are linked by short peptides whose amino acids may be of the D- or L-form and whose length and composition may vary depending

Fig. 4-13. Structure of the peptidoglycan of (A) a gram-positive bacterium (*Staphylococcus aureus*) and (B) a gram-negative bacterium (*E. coli*). Tetrapeptides are attached to NAM; a tetrapeptide from one chain can be connected to a tetrapeptide on an adjacent chain by a peptide bond, but some tetrapeptides do not produce interchain bridges. Arrows point to site of action of lysozyme. (NAM = N-acetylmuramic acid; NAG = N-acetylglucosamine; ⊥— = peptide bond; ● = tetrapeptide.)

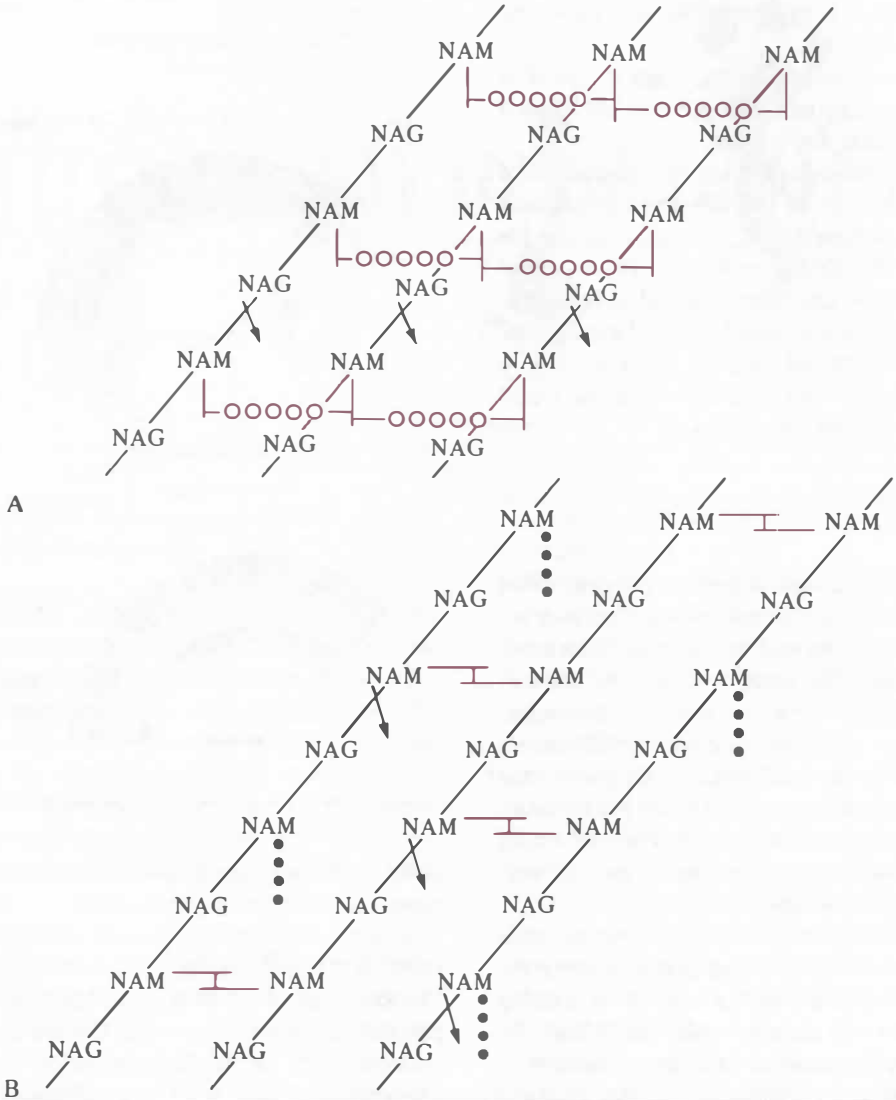
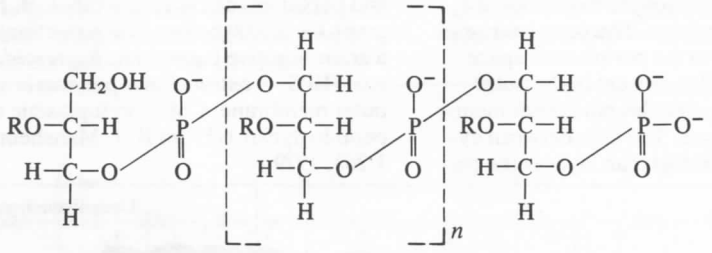
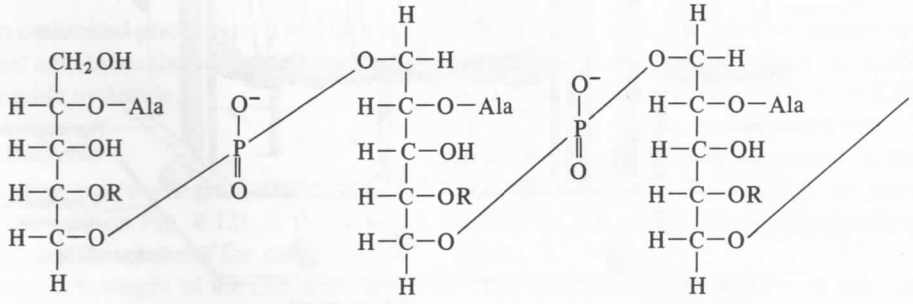


Fig. 4-14. Structure of teichoic acids. A. Glycerol teichoic acid. (R = H,D-alanine or glucose.) B. Ribitol teichoic acid. (R = N-acetylglucosamine or glucose; Ala = alanine.)



A



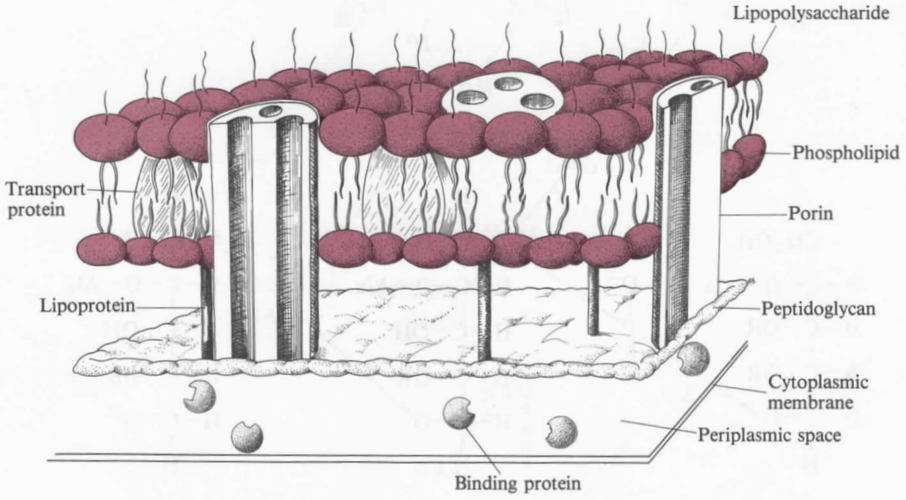
B

on the species. The peptidoglycan of *Staphylococcus aureus*, a gram-positive bacterium, is illustrated in Figure 4-13A. The degree of cross linkage in *S. aureus* and other gram-positive bacteria is very high, and thus the wall is more rigid than the walls of gram-negative species in which the amount of cross linking is very small (Fig. 4-13B).

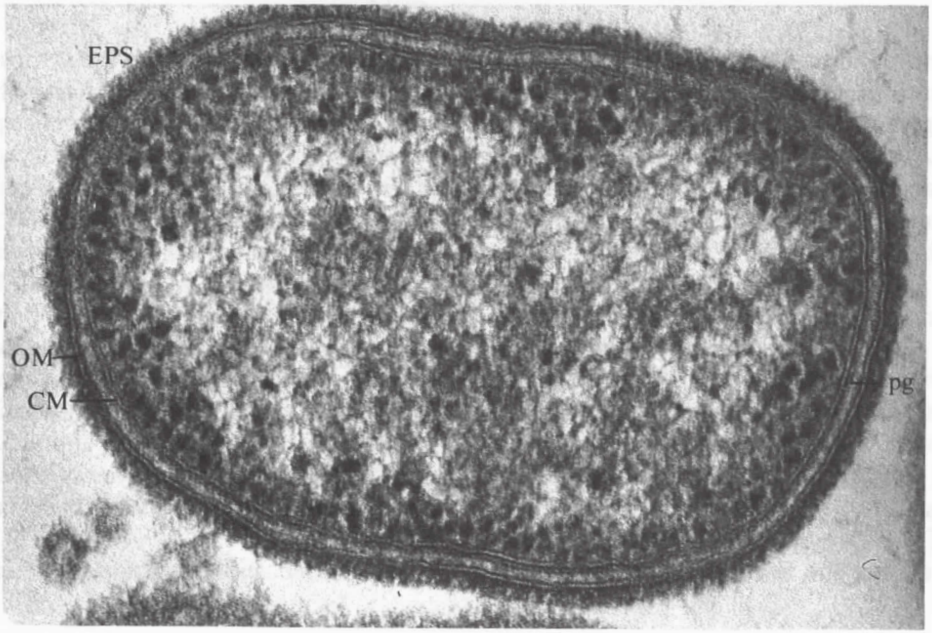
Teichoic acids are polysaccharides consisting of repeating units of glycerol or ribitol linked by phosphate (Fig. 4-14). This arrangement in the cell wall has yet to be clarified, but it is believed to form a branching network within the peptidoglycan and to be linked to the sugar residues in the peptidoglycan chain. The function of teichoic acids is unclear. They do provide a negative charge to the wall, because of the presence of phosphate groups, and could control the level of cations (positively charged ions) penetrating to the cytoplasmic membrane. Adsorption of some bacteriophages by *S. aureus* and strains of *Bacillus subtilis* appears to be dependent on the presence of glycosylated teichoic acids. Teichoic acid also appears to control the enzymes that degrade the cell wall. Sometimes the cell wall–degrading enzymes (autolysins) are engaged in total destruction of the cell, but at other times they are required to cleave portions of the cell wall so that new cell wall material can be inserted during growth. Teichoic acids are the principal surface structures on gram-positive bacteria that exhibit antigenicity. This antigenicity appears to be associated with the glycerol phosphate backbone and the degree of substitution of carbohydrate groups.

Teichuronic acids are produced when the bacte-

Fig. 4-15. Proposed structure of cell wall of gram-negative bacteria. A. Diagrammatic illustration of the gram-negative cell wall. Outer membrane consists of a sheet of lipopolysaccharide and phospholipid molecules in which are embedded porin proteins. The porins serve as channels for solute transport to the periplasmic space. Other transport proteins are also present in the outer membrane. The lipoprotein molecules bind outer membrane to the peptidoglycan layer. The area between cytoplasmic membrane and peptidoglycan layer is the periplasmic space, which contains various enzymes as well as binding proteins for solute transport. Not drawn to scale; for example, the space between outer membrane and peptidoglycan is exaggerated. B. Electron micrograph ($\times 60{,}000$ before 25% reduction) of thin section of a gram-negative bacterium, *Bacteroides melaninogenicus*. (EPS = extracellular polymeric substance; OM = outer membrane; CM = cytoplasmic membrane; pg = peptidoglycan.) (From B. J. Mansheim, *Rev. Infect. Dis.* 1:263, 1979.)

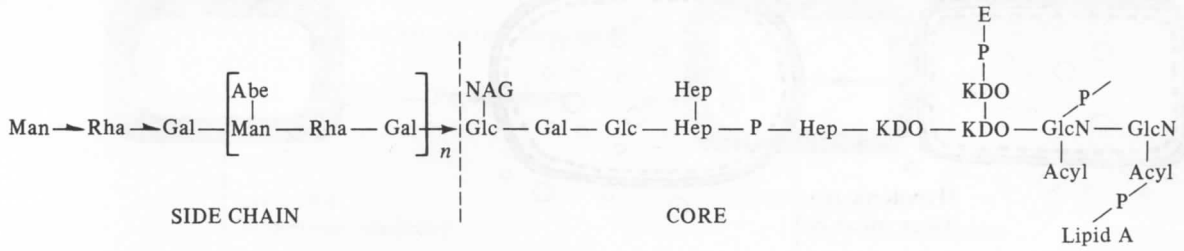


A



B

Fig. 4-16. Basic structure of lipopolysaccharide (LPS) found in gram-negative cell walls. The side chain consists of sugars that are responsible for the serological specificity of the organism. The core consists of sugars that remain relatively stable within a bacterial group. Lipid A is the toxic component of the LPS. (Man = mannose; Rha = rhamnose; Gal = galactose; Abe = abequose; Hep = L-glycero-D-mannoheptose; KDO = 2-keto-3-deoxyoctonic acid; E = ethanolamine; Glc = glucose; GlcN = glucosamine; Acyl = acetyl; P = phosphate; NAG = N-acetylglucosamine.)



rial cell is grown on limited phosphate. Thus uronic acid or glucuronic acid takes the place of phosphate in the polysaccharide molecule.

Gram-negative Bacteria

Unlike gram-positive cell walls gram-negative cell walls are multilayered (see Fig. 4-12). A thin layer of peptidoglycan, which accounts for only 5 to 10 percent of the total dry weight of the cell wall, lies outside the cytoplasmic membrane. External to the peptidoglycan is a complex *outer membrane* made up of phospholipid (20 to 30 percent), lipopolysaccharide (30 percent), and protein (40 to 50 percent). Between the peptidoglycan and the cytoplasmic membrane is an area referred to as the *periplasmic space*. The structure of the outer membrane is illustrated in Figure 4-15. The outer membrane consists of a lipid bilayer just like the cytoplasmic membrane, but the phospholipid layer closest to the environment also contains lipopolysaccharide (LPS). The LPS of gram-negative bacteria exhibits toxicity and is referred to as *endotoxin*. When endotoxin is produced and released by the cell it can cause fever and a multitude of other symptoms (see Chapter 12 for a discussion of endotoxin and disease). Endotoxin may be released when the cell wall lyses, or it may be released during vegetative growth. The lipid portion of the LPS is buried in the outer membrane and is referred to as *lipid A*, but the polysaccharide portions attached to it are at the cell surface. It is the lipid A moiety with which most of the biological activity of the LPS is associated. The polysaccharide portion of LPS is made up of a core region and a side chain. The basic structure of one LPS is illustrated in Figure 4-16. The outer membrane appears to be covalently attached to the peptidoglycan by lipoprotein molecules.

The polysaccharide portion of the LPS is very important because it is antigenic and can interact with host cells. These antigenic polysaccharides make up what is referred to as the somatic or O antigens of the cell. They do not vary in composition within a species and thus are very useful for identification purposes. They have been particularly helpful in the classification and identification of salmonellae. All members of the genus *Salmonella* have a common core of sugars, but each species has different O-specific side chains.

Proteins are also found on the outer membrane. The most important of these are called *porins*. Porins bridge the outer membrane and act as hydrophilic channels for transport of various molecules into the periplasmic space.

The periplasmic space acts as an osmotic barrier between the environment and the cell cytoplasm. Several enzymes located in this space process molecules before transport into the cytoplasm. Certain solute-binding proteins are also located in the periplasmic space.

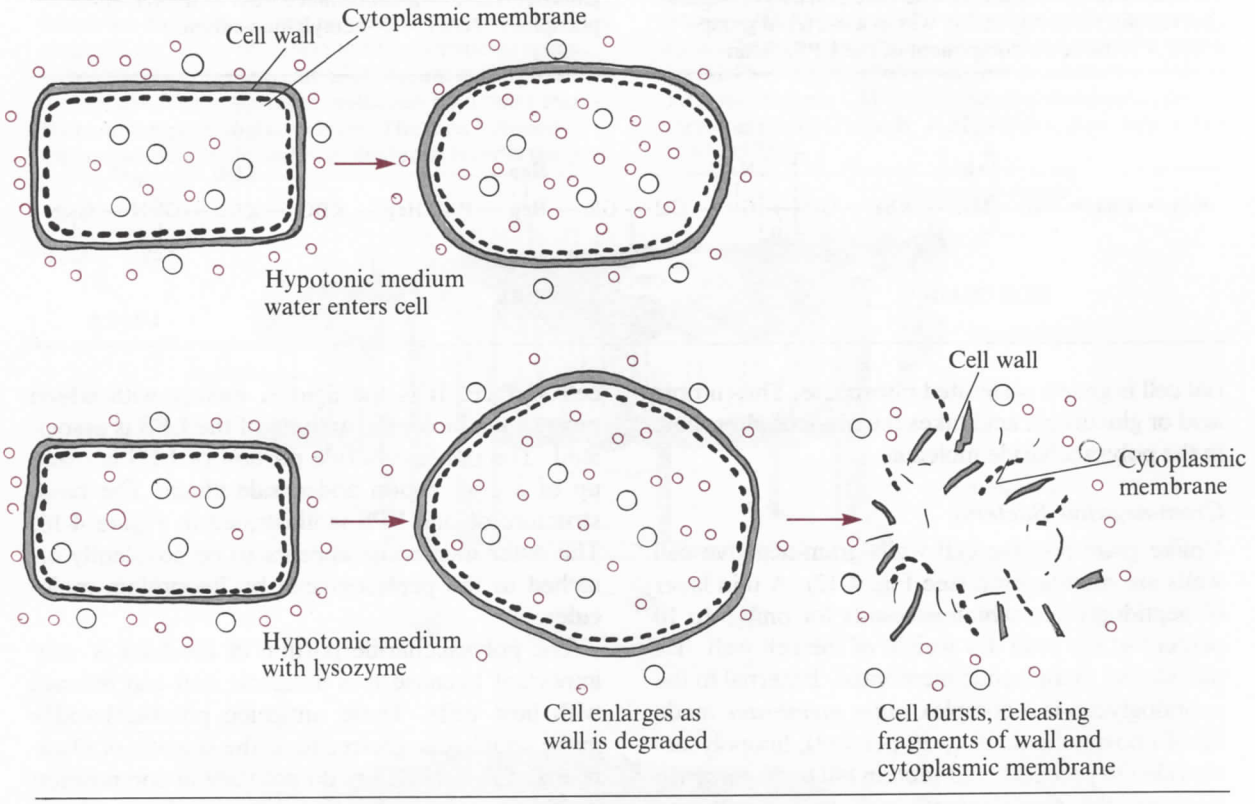


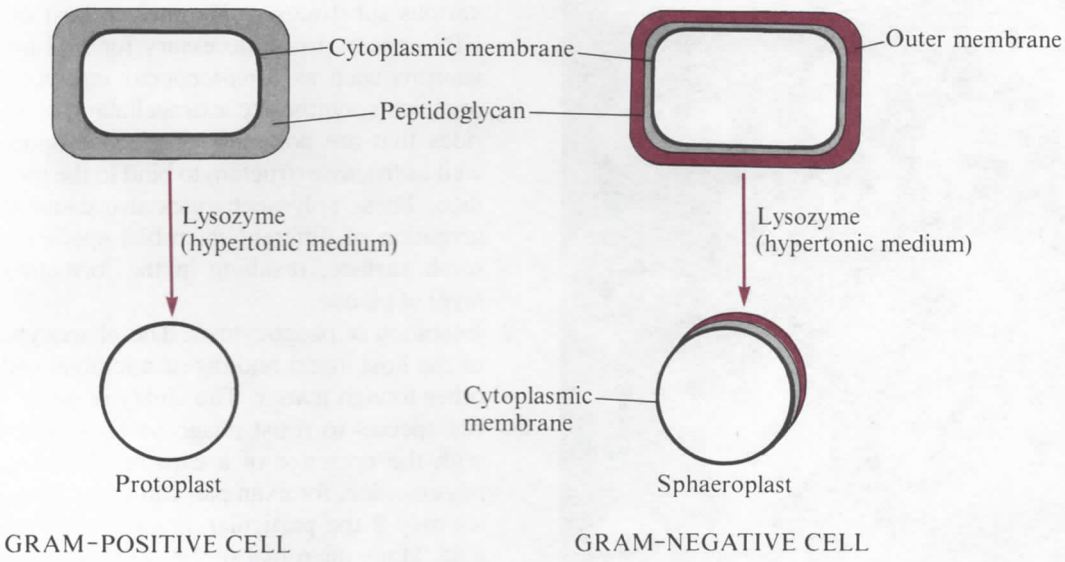
Fig. 4-17. Osmosis. A. Microbial cell in hypotonic medium takes water into cell but does not burst. B. Microbial cell in hypotonic medium containing lysozyme. As lysozyme degrades cell wall, water is taken into the cell, which causes it to expand and then burst. (○ = water molecule; ◯ = solute molecule.)

Osmosis and Cells Without Cell Walls

The cell wall is not a totally rigid structure but is capable of expanding and contracting. The solute concentration in the cell is usually greater than that outside the cell; therefore the cell is usually in a hypotonic environment. In a hypotonic medium water moves from the solution outside the cell into the cell cytoplasm to establish an equilibrium—a process called *osmosis* (Fig. 4-17A). The cell contents swell but do not burst because of the rigidity of the cell wall polymers. Without the cell wall the cytoplasmic membrane would expand and burst.

Sometimes in research it is necessary to remove the cell wall and produce a cellular unit that possesses an intact cytoplasmic membrane. This can be accomplished through the use of the enzyme lysozyme, which cleaves specific bonds in the peptidoglycan and by placing the cells in a hypertonic (high solute concentration) medium containing a solute such as sucrose. Once the wall has been digested, one of two types of intact cell membrane unit is produced: a *protoplast* if the original cell was gram-positive, or a *spheroplast* if the original cell was gram-negative (Fig. 4-18). Protoplasts are totally devoid of cell wall, but spheroplasts contain remnants of the cell wall. The protoplast or spheroplast does not burst because in a hypertonic me-

Fig. 4-18. Protoplast and spheroplast formation. A. Protoplast formation in gram-positive cell. Lysozyme removes entire cell wall. B. Spheroplast formation in gram-negative cell. Lysozyme does not remove all of the cell wall; some outer membrane remains.



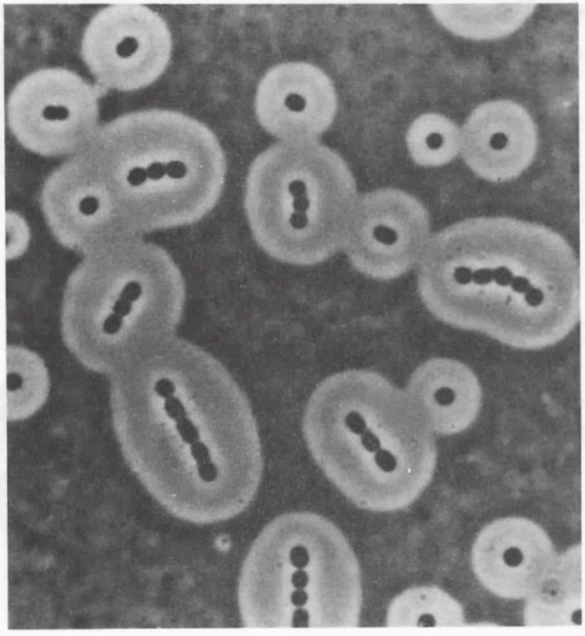
dium there is sufficient solute concentration outside the cell to counterbalance the solute concentration "pushing" against the cytoplasmic membrane from within the cell.

Protoplasts can be artificially produced in the laboratory, but there is some suspicion that they may also be produced during the treatment of certain infectious diseases with cell wall inhibitors. Antibiotics, such as penicillin or cephalosporin, inhibit cell wall synthesis (see Chapter 11). Treatment of an individual with penicillin may destroy most microorganisms, but those that survive could be accounted for by protoplast formation.

One genus of bacteria called *Mycoplasma* lacks a cell wall. They can survive without walls because their cytoplasmic membranes are fortified by the inclusion of sterols. These organisms grow very slowly and require special media for laboratory cultivation.

L-forms (named after the Lister Institute in London where they were discovered) are another group of wall-less microorganisms. They can be gram-negative or gram-positive cells that have lost their wall or part of their wall during growth in a host, but they can also be produced in the laboratory. L-forms differ in the amount of cell wall material that is attached to the cell. Those with no cell walls are incapable of producing new cell wall components. Those with cell wall remnants can produce new cell walls and sometimes are capable of reverting back to a stable walled form.

Fig. 4-19. Bacterial capsule. Light micrograph of wet mount of encapsulated bacteria. The capsules appear as halos around the cells in a background filled with India ink particles. (From W. H. Taylor and E. Juni, *J. Bacteriol.* 81:688, 1961.)



EXTRACELLULAR POLYMERIC SUBSTANCES

The bacterial cell may possess polymeric substances that are attached externally to the cell wall. These extracellular polymeric substances (EPS) are composed primarily of polysaccharides, but a few bacteria such as *Bacillus anthracis* have polypeptide EPS. EPS are considered a *capsule* if they exhibit some organization and adhere strongly to the cell wall. Loosely organized EPS that are not strongly attached to the cell wall are called *slime*. The EPS do not take up ordinary stains; therefore they can be demonstrated by suspending the bacteria in a solution containing colloidal particles such as India ink. The particles cannot penetrate the capsule; therefore the cell appears to have a halo (Fig. 4-19). EPS are not necessary for the survival of the cell, and continued cultivation of an organism in the laboratory may result in the eventual loss of EPS. The capsule can perform some functions that may play a role in disease:

1. Adherence. Soil and aquatic microorganisms use the capsule for adherence to other members of their species to form colonies or to adhere to various substrates. Adherence to host cells via EPS appears to be necessary for oral microorganisms such as *Streptococcus mutans*. These organisms synthesize extracellular polysaccharides that are polymers of glucose (glucan) as well as fructose (fructan) to bind to the tooth surface. These polysaccharides also cause the aggregation of different microbial species on the tooth surface, resulting in the formation of a layer of plaque.
2. Inhibition of phagocytosis. The phagocytic cells of the host ingest and digest microbial cells and other foreign matter. The ability of some bacterial species to resist phagocytosis is associated with the presence of a capsule. *Streptococcus pneumoniae*, for example, can resist phagocytosis only if the particular strain produces a capsule. Many microbial species produce a capsule, but it does not in all cases inhibit phagocytosis.
3. Antigenicity. Polysaccharide and protein EPS are antigenic and thus capable of stimulating an immune response. The capsular material from the major strains of *S. pneumoniae* has been used to produce a polyvalent* vaccine that has been used to prevent streptococcal pneumonia in individuals prone to the disease. Antigenicity is also used in the identification of capsule-producing bacteria. Strain-specific capsular antibody when applied to a culture of cells will cause the capsule to swell. The swelling is referred to as the *quellung reaction*.

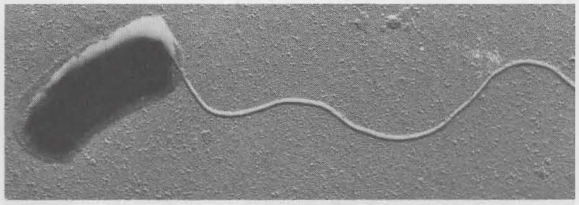
FLAGELLA AND OTHER STRUCTURES ASSOCIATED WITH MOVEMENT

Bacteria suspended in a solution can be observed microscopically to be moving in a random fashion.

**Polyvalent* in this context indicates that the vaccine contains several antigens capable of stimulating antibodies that react with different strains of *S. pneumoniae*.

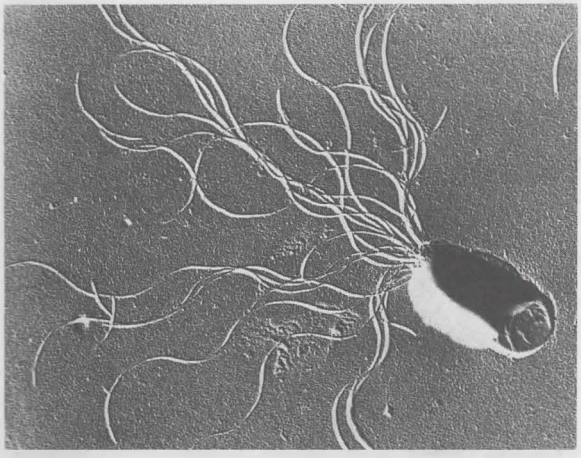
Fig. 4-20. Flagellar arrangement. A. Monotrichous. Electron micrograph (× 24,000 before 50% reduction). B. Lophotrichous. Electron micrograph (× 30,000 before 59% reduction). C. Amphitrichous. D. Peritrichous. Electron micrograph (× 24,000 before 44% reduction). (Electron micrographs courtesy W. Hodgkiss.)

Monotrichous



A

Lophotrichous



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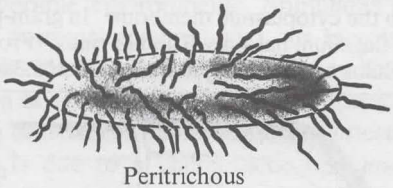
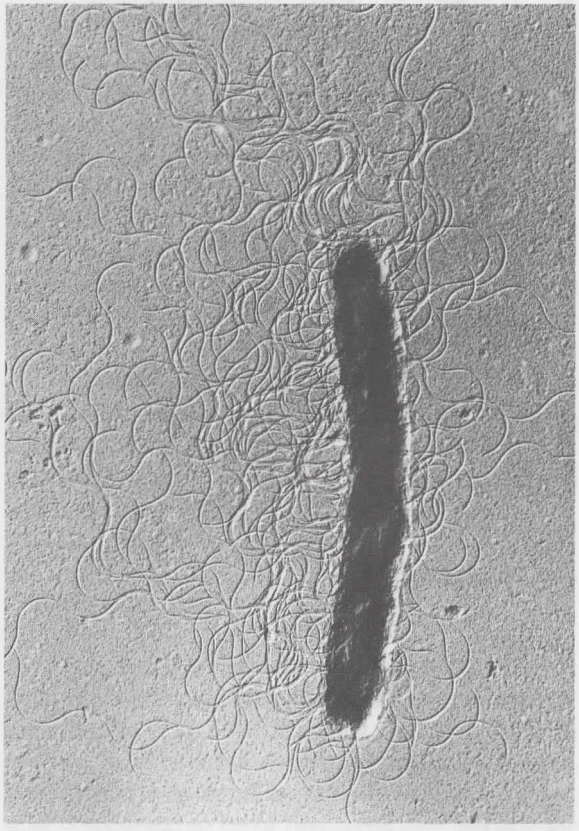


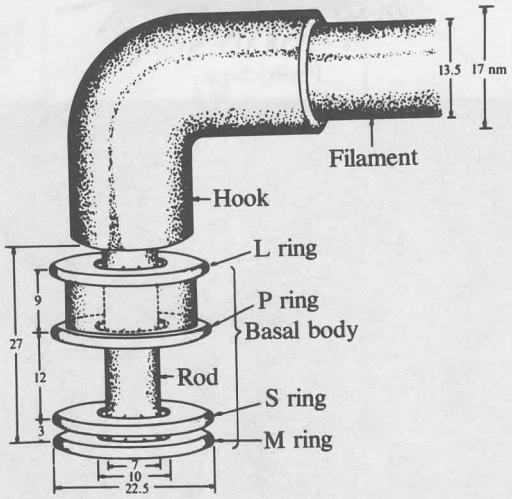
Peritrichous

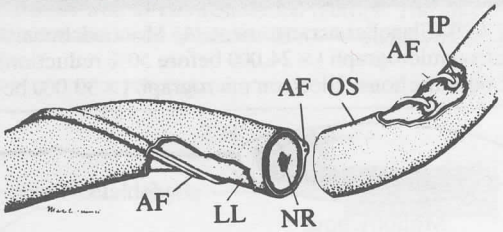


D

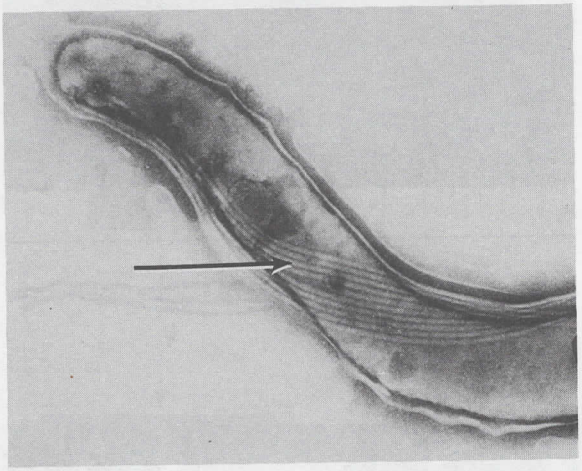
C Amphitrichous

Fig. 4-21. Prokaryotic flagellar structure. Flagellum consists of filament, hook, and basal body. In gram-negative cells the L and P rings are anchored to the outer membrane of the cell wall, and the S and M rings are attached to the cytoplasmic membrane. In gram-positive cells the flagellum has only S and M rings. (From M. L. DePamphilus and J. Adler, *J. Bacteriol.* 105:384, 1971.)





A



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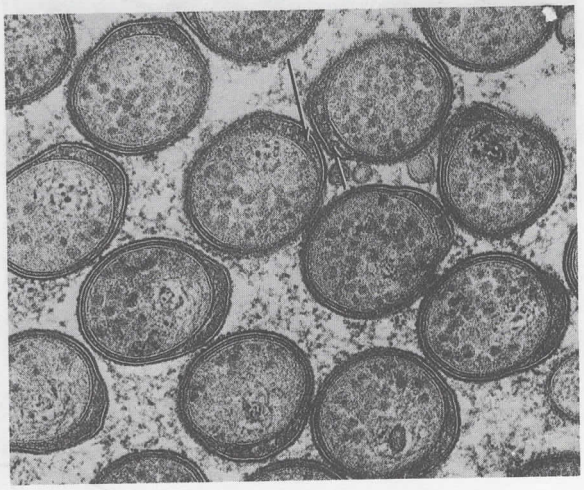


C

Fig. 4-22. Axial filaments. A. Relationship of the axial fibers in spirochetes to other cell components. Axial fibers (*AF*) are located between outer sheath (*OS*) and lipoprotein layer (*LL*) of the cell wall. Axial fibers are inserted into protoplasmic cylinder by insertion pores (*IP*). (NR = nuclear region.) (From S. Holt, *Microbiol. Rev.* 42:114, 1978.) B. Electron micrograph of axial filaments (*arrow*) as they appear to wind around the body of the spirochete. (From M. A. Listgarten and S. S. Socransky, *J. Bacteriol.* 88:1087, 1964.) C. Electron micrograph (×90,000 before 35% reduction) of cross section of spirochete showing the axial fibers (*arrows*) in the periplasmic space between outer sheath and inner membrane. (From K. Hovind-Hougen, *J. Clin. Microbiol.* 16:1127, 1982.)

This random movement is called *brownian movement* and is due to bombardment by water molecules on the surface of the cell. Many bacteria, however, are capable of independent and directed movement that is directly related to the presence of protein appendages called *flagella*. The protein in flagella is called flagellin. A cell may have a single flagellum or more than one, depending on the species (Fig. 4-20). The flagellum, which consists of a filament, hook, and basal body (Fig. 4-21), may range from 2 to 20 μm in length. The rings in the basal body differ between gram-positive and gram-negative bacteria, but their function remains the same—anchoring the flagellum to the cytoplasmic membrane and in some instances to the cell wall.

Flagella-like filaments called *axial filaments* are found in the group of bacteria called spirochetes. Axial filaments are chemically and structurally similar to flagella, but they do not emanate from the surface of the cell. Axial filaments are located between the body of the cell and an outer sheath (Fig. 4-22). Axial filaments are therefore not exposed to the external environment as are the flagella of other bacteria. Axial filaments are not hindered by the viscosity of the medium; thus spirochetes can move more rapidly through viscous media than can flagellated bacteria.

Flagella are not an essential requirement for survival of the cell, but they do provide certain advantages. The involuntary movement of an organism in response to stimuli is called *taxis*, or a *tactic response*. Tactic responses are absolutely dependent on the possession of motility. Taxes result in the accumulation of bacteria in physiologically favorable environments; they may also prevent accumulation in unfavorable environments. Stimulants of taxes include nutrients (chemotaxis), air (aerotaxis), or light (phototaxis). Recent evidence indicates that adhesion of certain bacteria, such as species of *Salmonella* and *Vibrio cholerae*, to the intestinal epithelium is due to a chemotactic response. Nonchemotactic mutants of these organisms adhere very poorly to the intestinal epithelium.

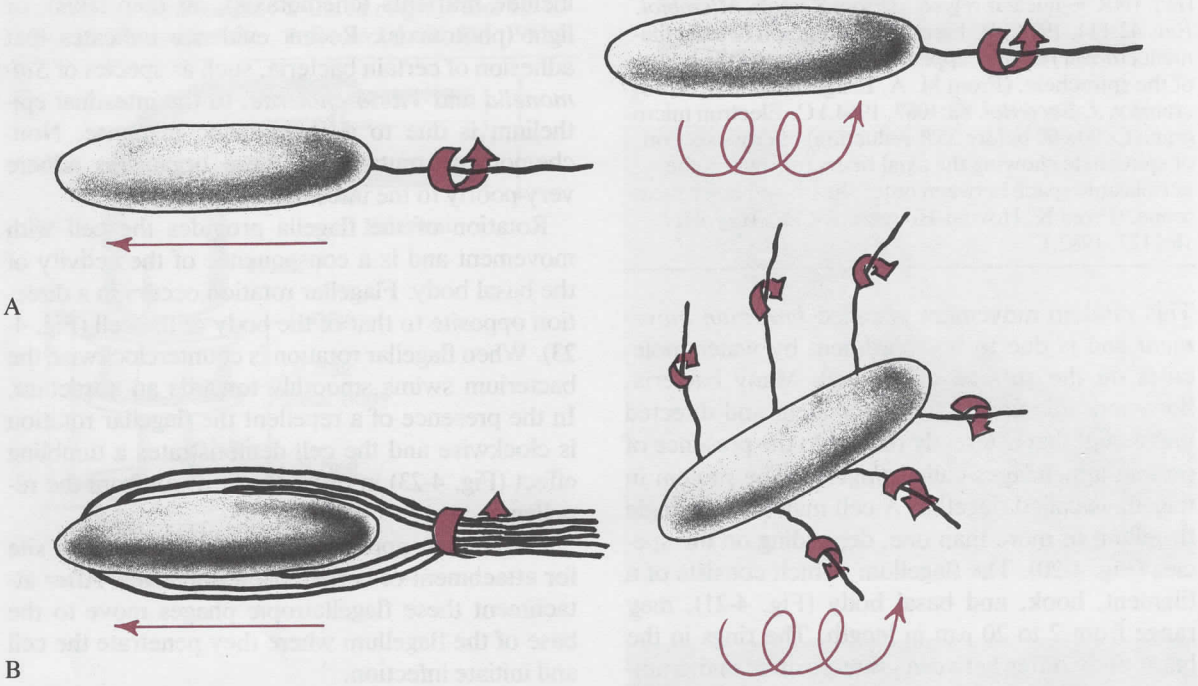
Rotation of the flagella provides the cell with movement and is a consequence of the activity of the basal body. Flagellar rotation occurs in a direction opposite to that of the body of the cell (Fig. 4-23). When flagellar rotation is counterclockwise the bacterium swims smoothly towards an attractant. In the presence of a repellent the flagellar rotation is clockwise and the cell demonstrates a tumbling effect (Fig. 4-23) in order to get away from the repellent.

Flagella are appendages that also provide a site for attachment of certain bacteriophages. After attachment these flagellatropic phages move to the base of the flagellum where they penetrate the cell and initiate infection.

PILI (FIMBRIAE)

Pili (sing. *pilus*) are protein appendages that project from the surface of the cell but are usually shorter than flagella (Fig. 4-24). Pili range in length from 30 to 200 nm. The term *fimbriae* frequently is used synonymously with *pili* by some in the field. Pili are now considered to be of two types, sex pili and attachment pili. Sex pili are found primarily on gram-negative bacteria and are involved in the transport of DNA between donor and recipient cell (see Chapter 5). Attachment pili, which are found in greater numbers on the surface of the cell than sex pili, are used for adhesion to various surfaces, including inanimate objects as well as various animal and plant cells. Adherence to plant and animal cells is often an important factor in the infectious disease

Fig. 4-23. Flagellar rotation. A. Rotation of a polar flagellum counterclockwise (*left*) results in smooth swimming with flagellum trailing cell. Clockwise rotation (*right*) causes cell to tumble with flagellum ahead of cell. B. In a peritrichously flagellated cell counterclockwise rotation causes flagella to form bundle and cell swims smoothly. When rotation of flagella is clockwise the flagella fly apart and cell tumbles.



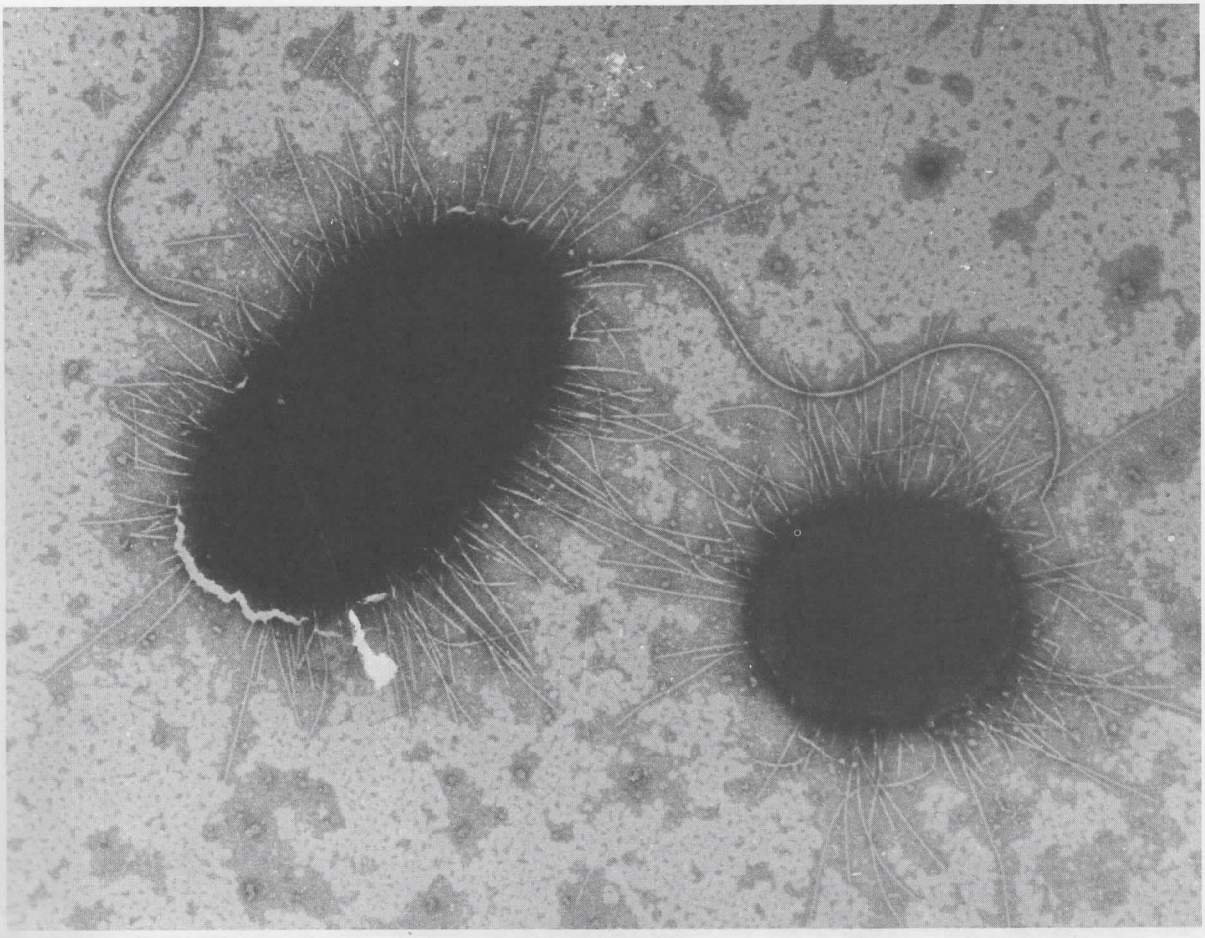
process (see Chapter 12). Attachment pili are found primarily on gram-negative bacteria, but some species of gram-positive bacteria, such as *Corynebacterium renale* and *Actinomyces naeslundii*, also possess them. Pili consist almost entirely of a protein called *pilin*.

STRUCTURES WITHIN THE CYTOPLASM

Nucleoids

Prokaryotes possess no nuclear membrane, but areas of DNA concentration, called *nucleoids*, can be observed in bacteria (Fig. 4-25). The prokaryotic DNA molecule is circular and is not associated with proteins. The DNA in bacterial cells such as *E. coli* can be carefully removed from the cell and when spread out is 1100 to 1400 μm long. DNA is, therefore, considerably condensed in the cell. Before cells divide, DNA is replicated while apparently attached to the cytoplasmic membrane in areas called *mesosomes* (see Fig. 4-5). Since each cell will receive a copy of DNA it is not surprising that the nuclear material can be observed at the center of the cell and site of cell division (septum). The number of nucleoids seen in the cell is usually dependent on the growth rate. At maximum rates of growth as many as four nucleoids can be observed in the cell, but only one nucleoid may be observed if the cell is not engaged in active growth.

Fig. 4-24. Electron micrograph (×30,000 before 20% reduction) of pili covering the surface of two bacterial cells. Compare their size with the long whip-like polar flagellum of the cell on the left. (Courtesy W. Hodgkiss.)



Ribosomes

Ribosomes are structures involved in protein synthesis in the cell. They are composed of approximately 60 percent RNA and 40 percent protein. When thin sections of bacteria are observed microscopically, ribosomes can be seen to fill the cytoplasm (Fig. 4-26). They may make up as much as 50 percent of the dry weight of the cell.

Inclusion Bodies (Intracellular Inclusions)

Bacterial growth under certain conditions can result in the accumulation of particles within the cytoplasm of the cell. These particles may or may not be covered with a membrane. Many of these inclusion bodies are observed in microorganisms found in the soil and water that are not involved in infectious diseases of humans. Gas vacuoles in aquatic bacteria, polyhedral bodies in organisms that use CO_2 as a sole source of carbon, and sulfur granules in bacteria that oxidize hydrogen sulfide are exam-

Fig. 4-25. Nucleoids. Micrograph of rod-shaped bacterium showing four areas of DNA concentration (*n*). (From A. C. Emeruwa and R. A. Hawirko, *J. Bacteriol.* 116:989, 1973.)

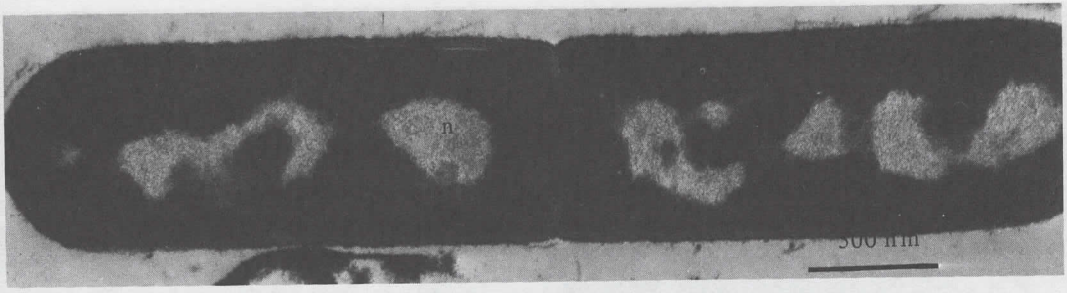


Fig. 4-26. Ribosomes. Electron micrograph of *Escherichia coli* demonstrating ribosomes (*R*), some of which are concentrated around nucleoids (*N*). (CM = cytoplasmic membrane; CW = cell wall.) (Courtesy A. Ryter, Institute of Pasteur.)

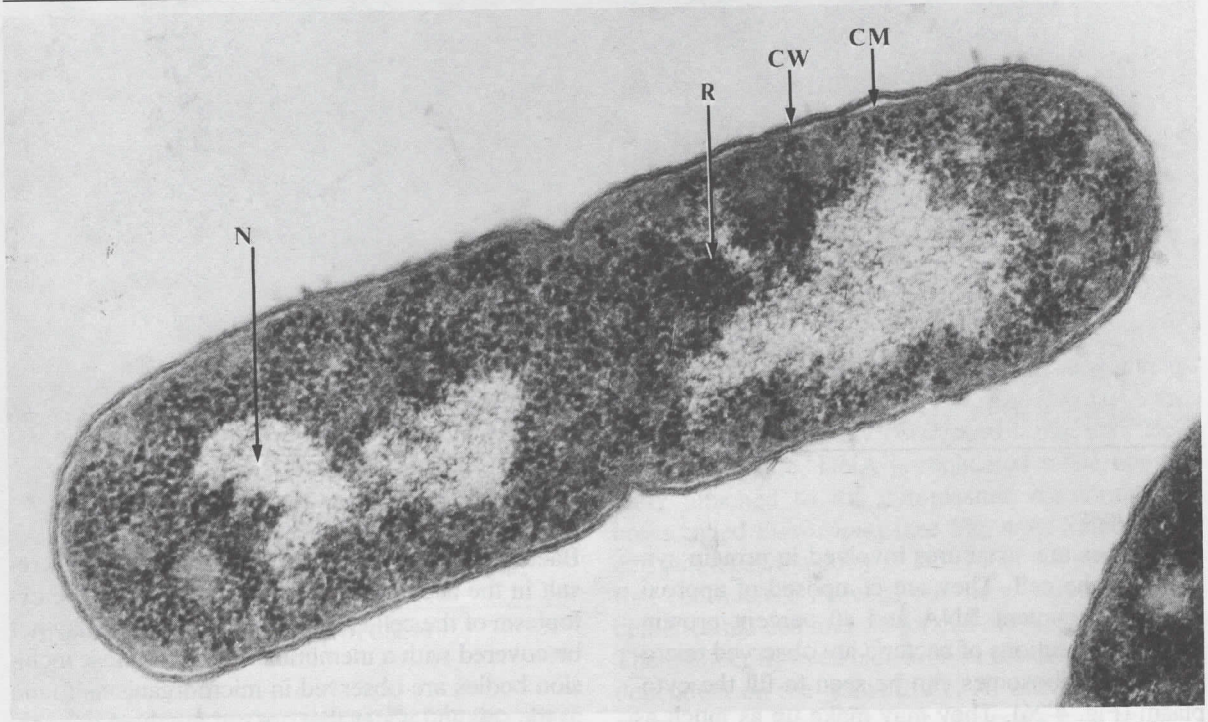


Fig. 4-27. Electron micrograph of glycogen granules (*arrow*) in a species of *Clostridium*. (From E. J. Laishley, *Can. J. Microbiol.* 19:991, 1973.)

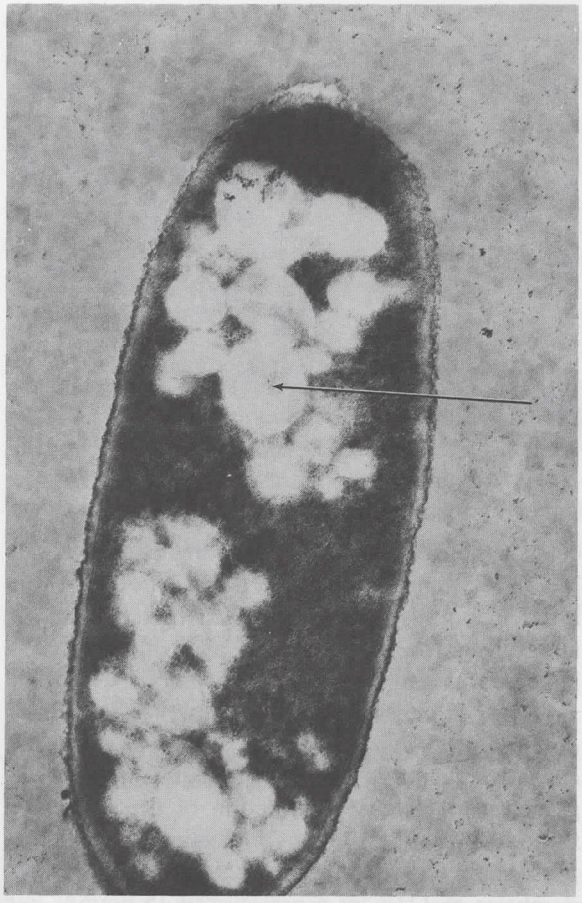
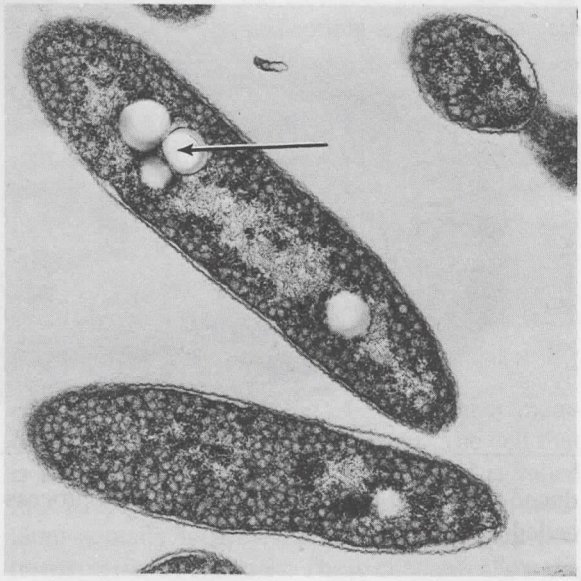


Fig. 4-28. Electron micrograph ($\times$ 43,000 before 23% reduction) of a species of *Rhodopseudomonas* containing membrane-bound inclusions of polyhydroxybutyrate (*arrow*). (From M. Madigan, et al., *J. Bacteriol.* 150:1422, 1982.)



ples of inclusions in bacteria that do not cause disease in humans.

Inclusions found in bacteria that infect humans are glycogen, lipid, and polyphosphate granules. Glycogen is a polymer of glucose that can be used as a source of energy or carbon or both by the cell (Fig. 4-27). Glycogen is surrounded by a membrane, but is is not a unit membrane. Lipid storage in the cell often takes the form of polyhydroxybutyrate granules, which are surrounded by a non–unit membrane (Fig. 4-28). Inorganic phosphate is stored by the cell in the form of polyphosphate granules. These granules are also called *volutin, metachromatic bodies,* or *Babes-Ernst bodies*.

Endospores

Cells that are engaged in active growth and reproduction are referred to as *vegetative* cells or as being in the state of *vegetative growth*. There are several groups of microorganisms, including bacteria, that under environmental stress (nutrient limitation and oxygen depletion, for example) are capable of ceasing vegetative growth and producing intracellular inclusions called *endospores*. The two most important genera of bacteria causing disease that produce endospores are *Bacillus* and *Clostridium*. Endospores, unlike vegetative cells, are usually resistant to heat, chemicals, and radiation. The endospore is a highly refractile body that is difficult to stain and can be readily observed microscopically (Fig. 4-29). Once environmental conditions are suitable for growth the endospore can be in-

Fig. 4-29. Electron micrograph (×76,250 before 23% reduction) of ultrathin section of sporulating *Clostridium bifermentans*. The spore is the white refractile body in the cell. (From P. D. Walker, *J. Appl. Microbiol.* 33:1, 1970.)

duced to return to the vegetative state in a process called *germination*.

SPORULATION PROCESS. During endospore formation the following events occur (Fig. 4-30): The chromatin material is extended along the entire length of the cell. The cell membrane invaginates, the DNA becomes compartmentalized, and a second membrane is formed around the unit, which can now be called a *forespore*. A peptidoglycan is synthesized and laid down between the two membranes of the forespore. This peptidoglycan layer, called the *cortex*, differs chemically from the peptidoglycan of the vegetative cell. High concentrations of calcium dipicolinate, which are spore-specific, are found within the area (core) surrounded by the cortex. Spore coats consisting of peptides impermeable to water are laid down around the cortex. When coat synthesis is completed, the vegetative portion of the cell (sporangium) lyses, releasing a free spore.

The free spore is highly resistant to heat, chemicals, and desiccation. Many theories have been proposed to explain this resistance. It has been suggested that dipicolinic acid, which makes up 12 percent of the dry weight of the spore, plays a major role in resistance to heat, but this has not been verified. It has also been suggested that the unique structure of the peptidoglycan layer of the spore is in some way associated with resistance.

Some of the principal sporeformers (*Clostridium botulinum, C. tetani*, and *C. perfringens*) are the causative agents of botulism, tetanus, and gas gangrene, respectively. Destruction of the spores of these agents is of obvious importance to medical personnel. The most resistant spores require temperatures of 121°C at a pressure of 15 pounds per square inch for 15 minutes to ensure their destruction.

GERMINATION. A spore may remain dormant for hundreds of years before becoming transformed into a vegetative cell. The transformation is related not to the age of the spore but to the need for certain environmental conditions. Before germination takes place, the spore must first be activated—for example, by heat in the presence of water, although other agents or conditions may also initiate activation. Once activated, the spore enters the stage of germination in which portions of the spore coats

become degraded. In the presence of growth-supporting nutrients the germinating spore swells and enters a period of biosynthesis in which the vegetative cellular components are produced. This period ends with release of the spore coat and the initiation of vegetative growth (Fig. 4-31).

CHARACTERISTICS OF EUKARYOTES

Eukaryotic microorganisms are more complex than prokaryotic microorganisms. Protozoa, fungi, and algae all possess the basic organelles and structures such as mitochondria, golgi, nucleus, endoplasmic reticulum, and in some cases chloroplasts. The structure and function of these components in the cell is reasonably invariant. The cell wall, cytoplasmic membrane, and other structures demonstrate considerable variation, depending on the microbial group and its particular habitat. We will concern ourselves primarily with the basic structures common to all eukaryotes. The morphological and biochemical properties of the individual groups of eukaryotic microorganisms will be discussed in later chapters. See Table 4-1 for a comparison of eukaryotes and prokaryotes.

CYTOPLASMIC MEMBRANE

The cytoplasmic membrane in eukaryotes is similar in structure and function to the prokaryotic membrane (see Cytoplasmic Membrane, earlier in this chapter). One of the principal differences between them is that sterols, which are complex alcohols possessing four ring structures, are present in eukaryotic membranes and not in prokaryotic cytoplasmic membranes, except in the *Mycoplasma*. Sterols are important in maintaining the stability of the membrane. For example, the protozoa are a group of microorganisms that do not possess a cell wall; the sterol-reinforced membrane maintains the cells' integrity. The susceptibility of certain eukaryotes to antibiotics is related to the presence of membrane sterols, as will be discussed in Chapter 11.

CELL WALL

Algae and fungi both possess cell walls that are chemically and structurally distinct from the prokaryotic cell wall. Peptidoglycan is not found in eukaryotic cell walls, and therefore this group of microorganisms is not susceptible to antibiotics such as penicillins and cephalosporins that inhibit bacterial cell wall synthesis. The fungal cell wall is 70 to 80 percent polysaccharide; the remainder is protein, with practically no lipid. The polysaccharides are chitin or cellulose, both of which are complex polymers of glucose that make the fungal cell wall much more rigid than the bacterial cell wall.

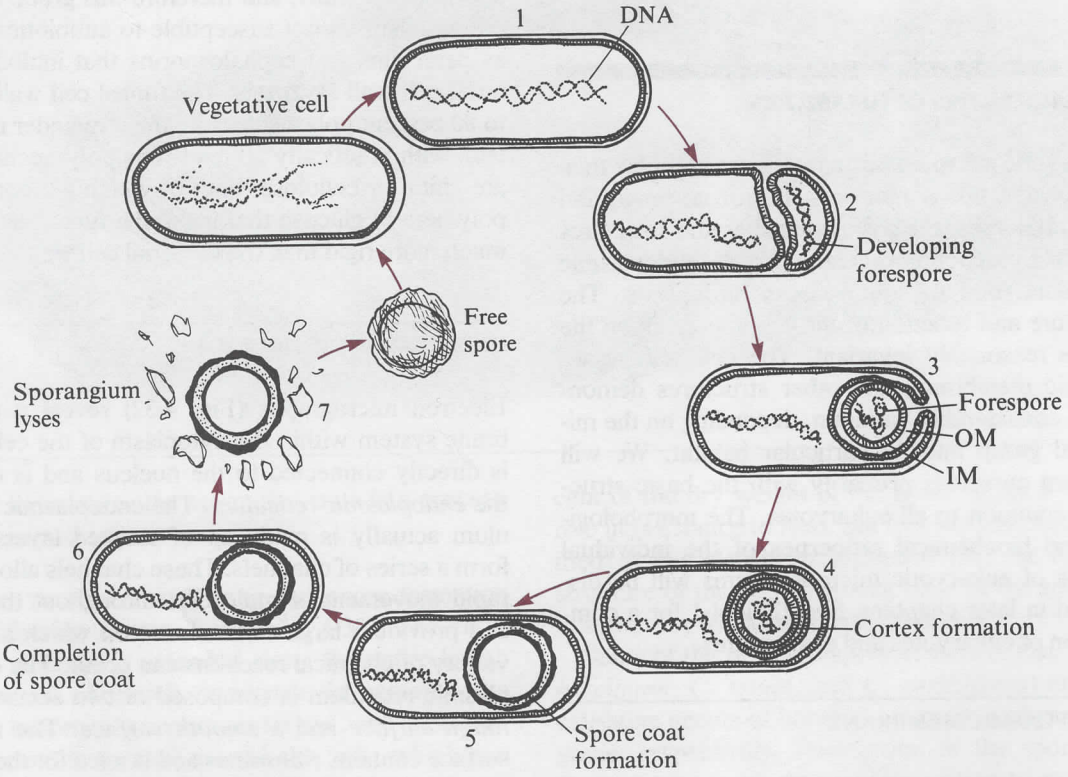
ENDOPLASMIC RETICULUM

Electron micrographs (Fig. 4-32) reveal a membrane system within the cytoplasm of the cell that is directly connected to the nucleus and is called the *endoplasmic reticulum*. The endoplasmic reticulum actually is made up of stacked layers that form a series of channels. These channels allow the rapid movement of molecules throughout the cell and provide a large surface area on which a wide variety of chemical reactions can occur. The endoplasmic reticulum is composed of two surfaces, a *rough surface* and a *smooth surface*. The rough surface contains ribosomes and is used for the synthesis of proteins. The smooth surface contains enzymes that are involved in lipid biosynthesis and in the biotransformation (detoxification) of drugs.

GOLGI

Golgi bodies are membranous (unit membrane) organelles whose functions are intimately associated with those of the endoplasmic reticulum. Golgi are composed of six or more flattened sacs (Fig. 4-33) from which pinch off small membranous structures. Proteins synthesized on the endoplasmic reticulum are passed to the Golgi in membranes. The proteins are processed in the Golgi, sometimes by the addition of various organic groups such as sug-

Fig. 4-30. Sporulation process. A. Diagram. B. Electron micrograph of sporulation. (1) Vegetative cell. (2) Accumulation of storage granules (*G*). (3) Formation of forespore septum (*FS*). (4) Enlargement of forespore (*F*) and spore coat (*C*) digestion. (5) Cortex (*Cx*) formation and spore maturation. (6) Mature spore (*S*) with exosporium (*E*). (7) Detail of spore structure. (IC = inner coat; OC = outer coat.) (From S. Long, D. T. Jones, and D. P. Woods, *Appl. Environ. Microbiol.* 45:1389, 1983.)



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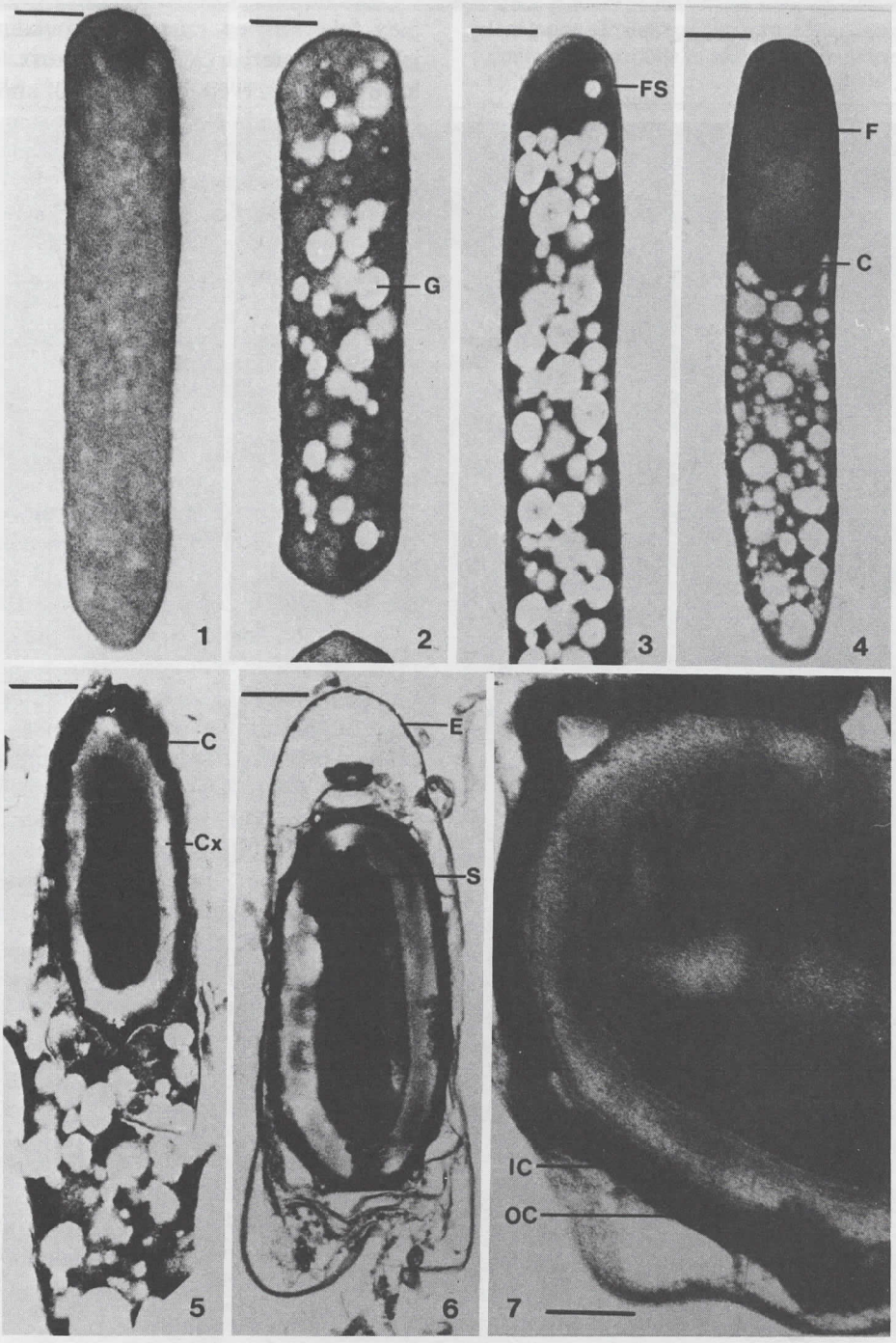
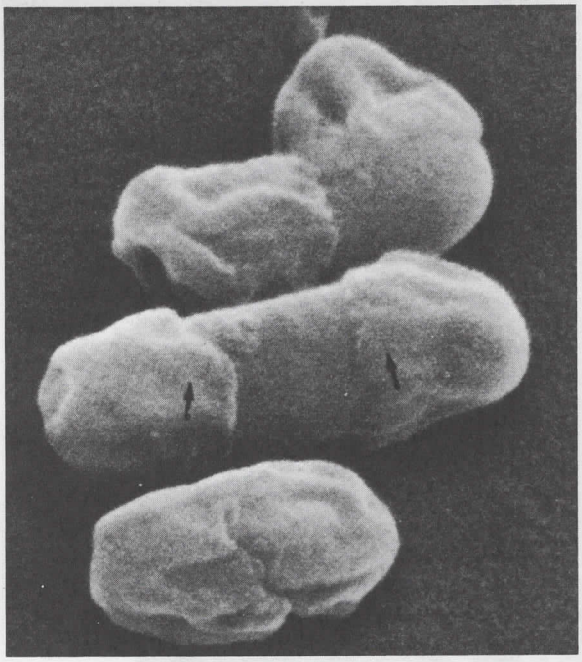


B

Fig. 4-31. Scanning electron micrograph of spores in the process of germination and loss of spore coat (*arrows*). (Courtesy Z. Yoshii.)



ars (glycoproteins) or lipids (lipoproteins) or of inorganic groups such as sulfate. These modified proteins are enclosed in a membrane within the Golgi that pinches off to form a *vesicle*. The vesicles are transported to those areas of the cell where they are needed. A specialized vesicle pinched off from the Golgi is called a *lysosome*. The lysosome contains a number of degradative enzymes that are used to degrade cellular components or foreign bodies. Lysosomes are discussed further in Chapter 14.

MITOCHONDRIA

Mitochondria are double-membraned organelles that are involved in aerobic respiration (Fig. 4-34). The mitochondrion is composed of a complex set of enzymes most of which are located on the inner membrane. The inner membrane appears as complex folds, called *cristae*, surrounding a central jelly-like material called the matrix. The cristae have been observed to be made of knob-like units. Cristae engage in electron transport and adenosine triphosphate (ATP) production, while the matrix contains enzymes that engage in the tricarboxylic acid cycle reactions (these reactions provide protons for electron transport and precursors for amino acid synthesis).

FLAGELLA, CILIA, AND CYTOPLASMIC STREAMING

Movement in eukaryotic cells that are infectious for humans may require flagella or cilia or may be a specialized type of movement called cytoplasmic streaming. Movement involving flagella and cilia is found primarily in the protozoa. Flagella and cilia are appendages that emanate from the cytoplasm and project from the surface of the cell. The two types are chemically similar but differ in size and number per cell. Cilia, for example, are shorter and more numerous than flagella on eukaryotic cells. A protozoan called *Paramecium* contains over 10,000 cilia (Fig. 4-35). Flagella and cilia of eukaryotes are structurally different from prokaryotic flagella and are composed of protein units called *microtubules*. In cross section cilia and flagella have a pattern of two central microtubules encircled by nine doublet microtubules (Fig. 4-36). The doublets are extensions of the basal body. The basal body is derived from the centriole, the organelle that is involved in nuclear division.

Cytoplasmic streaming is a characteristic of all eukaryotic cells, but in the wall-less protozoa called *amoebae* it produces a type of movement called *amoeboid movement*. The movement of the cytoplasm forces the cell membrane to produce projections called *pseudopodia*. On a solid surface the formation of pseudopodia enables the organism to move (Fig. 4-37).

Fig. 4-32. Electron micrograph of a eukaryotic cell revealing endoplasmic reticulum (*ER*) closely associated with the nucleus. Arrows point to nuclear pores. (From D. W. Fawcett, *Cell Biology*. Philadelphia: Saunders, 1977.)

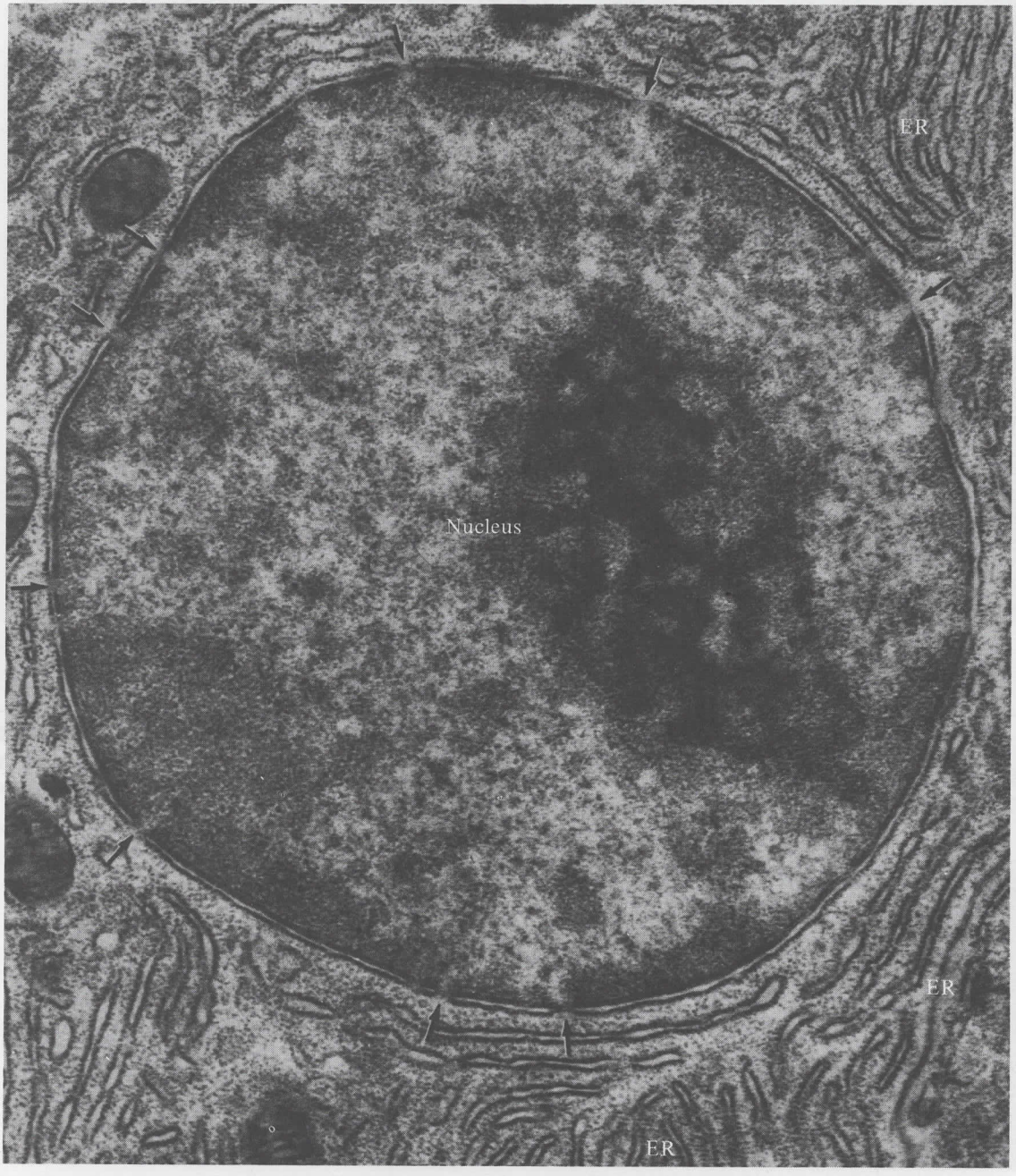


Fig. 4-33. Electron micrograph demonstrating the Golgi complex. (From D. W. Fawcett, *Cell Biology*. Philadelphia: Saunders, 1977.)

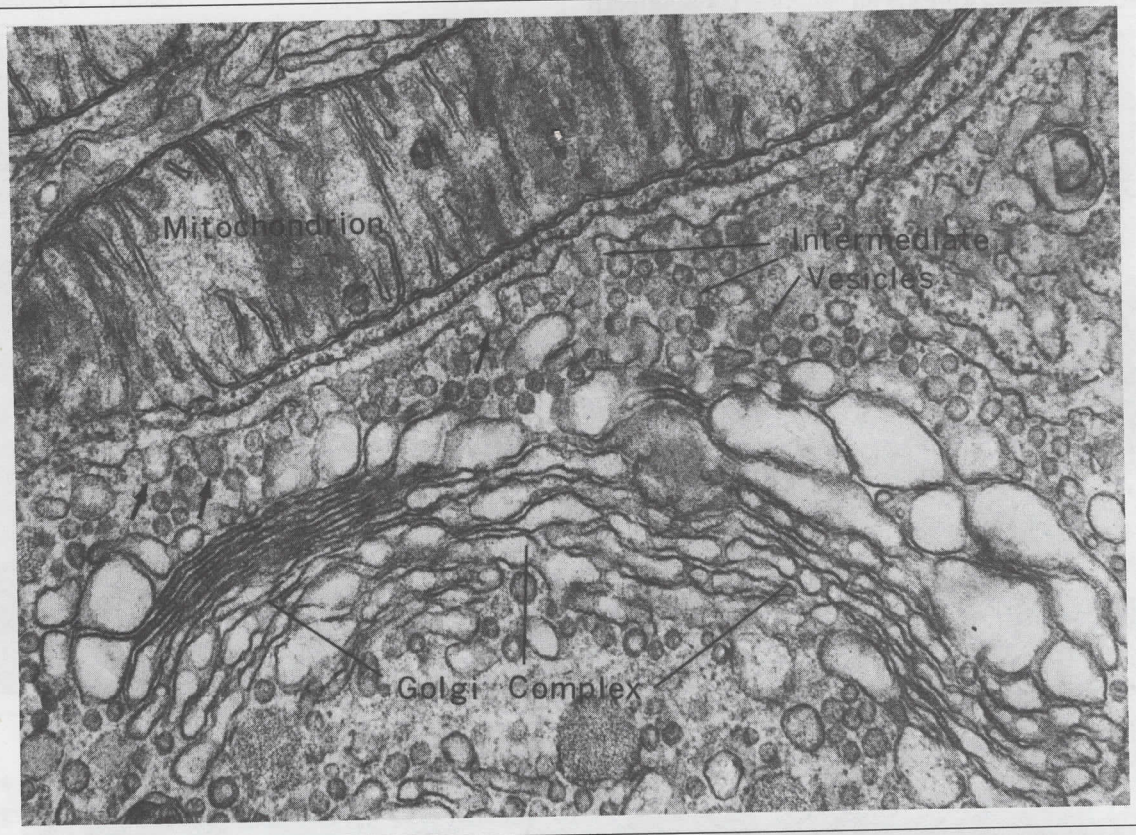
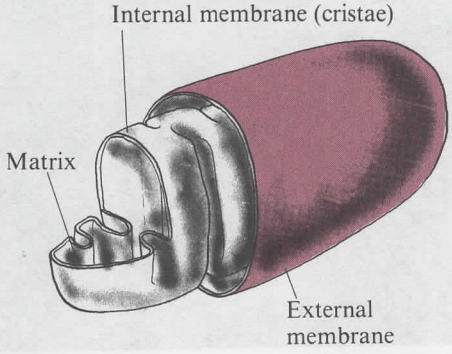


Fig. 4-34. Mitochondrion. A. Diagram. B. Electron micrograph. (From D. W. Fawcett, *Cell Biology*. Philadelphia: Saunders, 1977.)



A

B

Fig. 4-35. Scanning electron micrograph of *Paramecium caudatum*, preserved in a state of movement, demonstrating the direction of movement of the cilia. (From D. I. Barlow and M. A. Sleigh, *J. Microsc.* 115:81, 1979.)

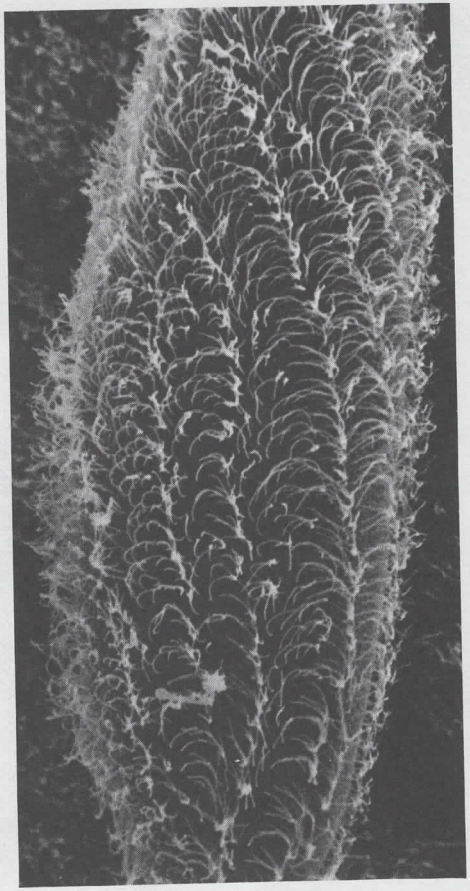
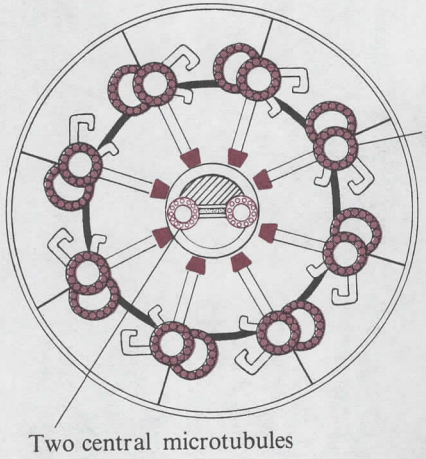


Fig. 4-36. Eukaryotic cilia. A. Diagram of the cross section of a eukaryotic cilium. Two central microtubules are surrounded by nine doublet microtubules. B. Electron micrograph of cross section of a cilium. (From E. Freymuller and E. P. Camargo, *J. Protozool.* 28:175, 1981.)



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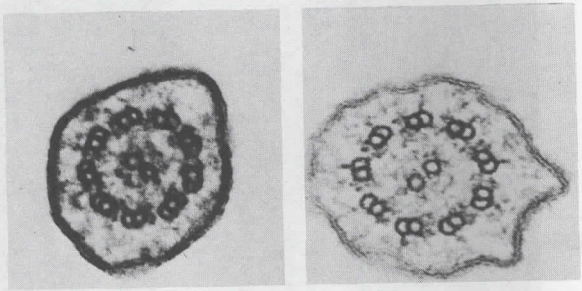

B

Fig. 4-37. Cytoplasmic streaming. Arrows show direction of cytoplasmic flow in the ameba.

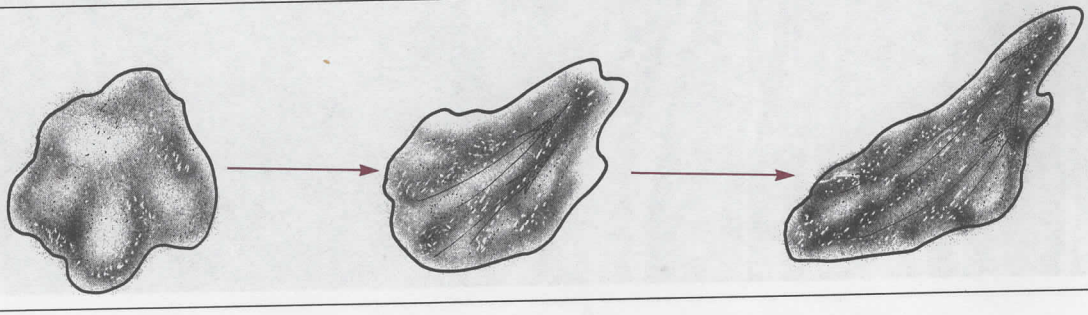

Fig. 4-38. Electron micrograph of eukaryotic cell demonstrating several cytoplasmic components including nucleus, within which is located the nucleolus. (ER = endoplasmic reticulum; M = mitochondrion; CM = cytoplasmic membrane; NE = nuclear envelope.) (From R. J. Szollosi and D. Szollosi, *Atlas of Cell Biology*. Boston: Little, Brown and Co., 1977.)

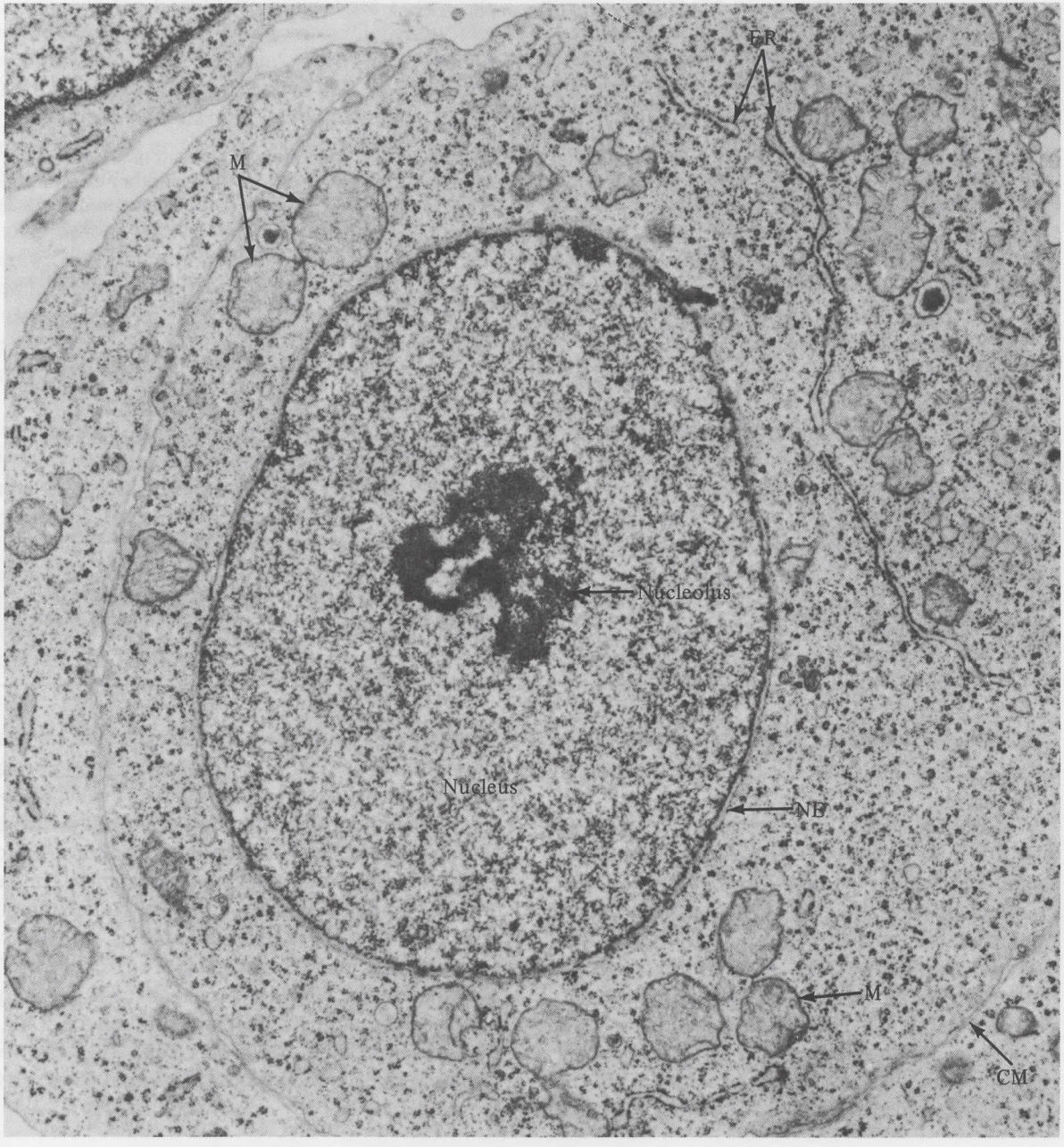

Table 4-3. Summary of Prokaryotic Structure and Function

Structure	Chemical composition	Function
Cell wall		Cell shape and protection of cytoplasmic contents
Gram-positive	Mainly peptidoglycan plus teichoic acid	—
Gram-negative	Very little peptidoglycan; outer membrane multilayered, containing phospholipid, lipopolysaccharide, and protein	—
Cytoplasmic membrane	Phospholipid bilayer containing globular proteins	Controls movement of solutes into cell; site of respiratory enzymes; secretes cytoplasmic products; contains enzymes for cell wall synthesis; site of DNA synthesis
Extracellular polymeric substances	Single polysaccharides; mixed polysaccharides, or proteins	Adherence to substrates, inhibition of phagocytosis
Flagella	Protein (flagellin)	Movement
Pili	Protein (pilin)	Conjugation in gram-negative bacteria; adherence to inanimate and animate surfaces
Nucleoid	DNA	Carries hereditary information
Ribosomes	RNA and protein	Protein synthesis
Inclusion bodies		
Glycogen	Polysaccharide (glucose)	Storage form of energy
Polyhydroxybutyrate	Lipid	Storage form of energy
Volutin (metachromatic granules)	Polyphosphate	?
Endospores	Complex peptidoglycan unit containing calcium dipicolinate; a dehydrated form of the vegetative cell	Protection from environmental stresses of a chemical or physical nature

NUCLEUS

The DNA of eukaryotes is enclosed in a porous membrane called the *nuclear membrane*. More complex than the membrane surrounding various organelles, the nuclear membrane is composed of two unit membranes. The outer membrane of the nuclear membrane is continuous with the endoplasmic reticulum at several sites. The pores in the nuclear membrane allow the transport of various molecules as well as ribosomal components that are produced in the nucleus but assembled in the cytoplasm. The DNA of the eukaryote appears microscopically as threads or filaments called *chromatin* when the cell is not engaged in cell division. During cell division the chromatin condenses into discrete rod-like units called *chromosomes*. Unlike prokaryotic DNA, eukaryotic DNA is a linear molecule and is associated with proteins called *histones* (see Chapter 5).

Within the nucleus is an area that stains intensely because it contains high concentration of RNA. This area is called the *nucleolus* (Fig. 4-38). The nucleolus serves as the site for ribosomal RNA synthesis.

SUMMARY

The basic distinction between prokaryotes and eukaryotes is the presence of a nuclear membrane surrounding the hereditary information in eukaryotes and its absence in prokaryotes. Generally, eukaryotes are more complex than prokaryotes. These distinctions are outlined in Table 4-1.

Classification of bacteria for many years was primarily artificial and based on a chemotaxonomic approach. In recent years natural classification based on the evolutionary history of the macromolecules DNA, RNA, and protein has developed. *Bergey's Manual of Determinative Bacteriology* was at one time the primary source for the classification of microorganisms, but that manual is now considered merely a handbook for the identification of bacteria.

Bacteria are divided primarily into two groups, gram-positive and gram-negative, based on the chemical composition of the cell wall. Other structures in the bacterial cell can be found in both groups and are outlined in Table 4-3.

Some eukaryotes, such as fungi and algae, have cell walls, but the protozoa do not. The cell walls of eukaryotes differ considerably from the cell walls of bacteria in architecture and chemical composition. The cytoplasmic membrane of eukaryotes is similar to that of bacteria, but eukaryotes also possess a channeled membrane system called the endoplasmic reticulum (ER). The ER channels nutrients throughout the cytoplasm and is connected to the nuclear membrane. One portion of the ER, called the rough ER, contains ribosomes, which are used in protein synthesis. The Golgi are cytoplasmic bodies engaged in the formation of membranous units called vesicles, which can be modified to carry various degradative enzymes. The Golgi also process various proteins produced by the ER as well as lipids and carbohydrates. Mitochondria are small bodies in the eukaryotic cell that carry out the respiratory activities normally associated with the cytoplasmic membrane in bacteria. Eukaryotes can move by means of flagella, cilia, or cytoplasmic streaming. Flagella and cilia are chemically and structurally similar, but they differ in size. They are also different from prokaryotic flagella. Eukaryotes have a distinct nucleus and nuclear membrane within which is located the DNA. The nuclear membrane is porous with connections to the endoplasmic reticulum. The DNA of eukaryotes is a linear molecule associated with proteins called histones. The DNA appears as thread-like units called chromatin when the cell is not engaged in division. During division the DNA condenses and forms rod-like bodies called chromosomes.

QUESTIONS FOR STUDY

1. What techniques are available for the phylogenetic classification of bacteria?
2. What is one of the best sources that identifies bacteria by a chemotaxonomic method?
3. What are the archaebacteria? How are they different from the eubacteria?
4. What is meant by the term *strains of a species*?
5. Why is morphology not an appropriate means of classifying bacteria?
6. Describe some conditions that can lead to pleomorphism in bacteria.
7. How do gram-negative and gram-positive cell walls differ in chemical composition?
8. What are the functions of the cytoplasmic membrane in bacteria? Are they the same as those in eukaryotic cells?
9. What is a protoplast? A spheroplast?
10. How does the cell secrete proteins?

11. Describe the structural arrangement of the outer membrane of gram-negative bacteria. Which part is toxic? Which part is antigenic?
12. How do *Mycoplasma* survive without cell walls?
13. What is the distinction between a capsule and slime layer? What functions do they have?
14. What is the difference between prokaryotic and eukaryotic flagella?
15. What is meant by a chemotactic response? How do bacteria respond to various stimulants?
16. What are pili? What functions do they serve for the bacterial cell?
17. What chemical component present in the endospore is absent in the vegetative cell?
18. Which genera of bacteria are associated with endospore formation?
19. What is the function of the endoplasmic reticulum? What is meant by rough and smooth endoplasmic reticulum?
20. Where do lysosomes come from? What is their function in the cell?

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5. GENETICS: STORAGE AND TRANSFER OF GENETIC INFORMATION

OBJECTIVES

To describe the steps involved in the replication of a double-stranded molecule of DNA

To describe the steps involved in the transcription of DNA to a messenger RNA molecule

To briefly list the steps associated with the translation of messenger RNA into a protein

To describe the operon model for control of protein synthesis

To describe what is meant by the genetic code and how mutations can affect it

To describe how mutants can be isolated

To describe the Ames test for detecting potential chemical carcinogens

To distinguish the three methods of DNA transfer: transformation, transduction, and conjugation

To describe the role of plasmids in the life of a bacterial cell

To explain the technique of genetic engineering and its significance

To define: genotype, phenotype, plasmid, codon, anticodon, inducible, constitutive, insertion sequence, transposon, photoreactivation repair, auxotroph, prototroph, sex factor, colicin, cloning, exon, intron

DNA Structure and Function
Characteristics of Microbial Chromosomal DNA
DNA Replication
DNA Transcription
DNA and the Genetic Code
Translation of the Genetic Code (Protein Synthesis)
Amino Acid Activation
Initiation
Elongation
Termination
Control of Protein Synthesis
Induction
Operon
Inducible vs. Repressible Operons
DNA and Mutation
Spontaneous Mutations
Induced Mutations
Effect of Mutation on the Genetic Code
Mutant Isolation and Detection
Isolation
Detection of Chemical Carcinogens
DNA Transfer and Recombination
Transformation
Conjugation
Hfr Cell Formation
Transduction
Characteristics of Extrachromosomal DNA (Plasmids)
Characteristics of Plasmids
Plasmid Compatibility
Characteristics of Some Important Plasmids
Bacteriocin Production
Antibiotic Resistance Factors
Catabolizing Activity
Plant Hyperplasia
Resistance to Metal Ions
Virulence Determinants
Other Accessory DNA Elements
Insertion Sequences
Transposons
Restriction Endonucleases and Recombinant DNA
Importance of Recombinant DNA Technology
Eukaryotic Genetics
Eukaryotic DNA Function
Eukaryotic Processing of RNA

The traits of an organism, whether it is a microorganism or a human, are encoded in the hereditary material called *DNA*. A student might think that a discussion of microbial genetics and how the microorganism stores and transfers genetic information would be boring and hardly worth the effort. Quite the contrary. The rapid development of molecular genetics, in conjunction with the introduction of recombinant DNA technology, has elicited considerable interest among scientists, physicians, and the public in general. Our ability to manipulate the hereditary material of lower organisms and even of our own species has far-reaching consequences.

The purpose of this chapter is to discuss the functions of the hereditary material in microorganisms and to examine how manipulation of the genetic material is propelling this generation and succeeding generations into areas of public as well as scientific concern.

DNA STRUCTURE AND FUNCTION

The structure of DNA was discussed in Chapter 2, and we suggest that the student review the material in that chapter before continuing this chapter.

The bacterial chromosome is a circular double-stranded molecule that is twisted on itself within the cell. The molecular weight of the *Escherichia coli* chromosome is approximately 3×10^9 daltons. There are several hundred thousand nucleotides in a single DNA strand, and considering that 0.34 nm is the distance between the nucleotide bases, the

DNA molecule could not be incorporated into the cell without folding and twisting. The DNA molecule carries the determinants of heredity in specific nucleotide sequences called *genes*, which altogether make up the *genotype* of the cell. The translation of these genes leads to the expression of specific traits called the *phenotype*. The DNA molecule of the cell that carries the genes without which the cell cannot exist is called *chromosomal DNA*. The survival of the species is dependent on the duplication of the chromosomal DNA and its separation into daughter cells at cell division. Some bacteria also carry *extrachromosomal DNA*, called *plasmid DNA*, which confers certain advantages to the cell over other bacterial cells but is not required for its survival.

The DNA molecule is involved not only in replication and transfer to progeny cells during cell division but also in activities that influence the phenotypic properties of the cell and even other cells. These activities include mutation, transfer of DNA to other cells in processes other than cell division, translation of genetic information into proteins, and control of protein synthesis.

CHARACTERISTICS OF MICROBIAL CHROMOSOMAL DNA

DNA REPLICATION

The bacterial chromosome contains several hundred thousand nucleotides, and the transmission of this genetic material from parent to progeny requires an exact duplication of the two DNA strands. Major errors in the duplication process could be disastrous, leading to conditions that prevent normal cell growth and development.

The bacterial chromosome is in a supercoiled state in the cell and must be unwound to be duplicated. In vitro experiments performed nearly 25 years ago demonstrated that the DNA molecule, before cell division, replicates in a semiconservative manner. Each strand of the parent DNA serves as a template for the formation of a complementary strand (Fig. 5-1). How the DNA molecule unwinds in the cell and performs this duplication process is still under investigation, but it is believed to occur in the following way (Fig. 5-2):

1. The bacterial chromosome possesses a site for unwinding and DNA replication referred to as the *origin*. It is recognized and activated by specific proteins. Some of these proteins are unwinding proteins, helix-destabilizing proteins, and DNA gyrase. Unwinding proteins produce local denaturation and cause the helix to unwind. Helix-destabilizing proteins bind to single strands and stabilize the strands as they are being replicated. DNA gyrase reduces the tension produced by double-strand breaks.

2. DNA synthesis is catalyzed by the enzyme *DNA polymerase* with short RNA primers being part of the process. Once the DNA molecule has been unwound, short RNA primers that are complementary to each template DNA strand are synthesized. Deoxyribonucleotides are added to the 3′ end of the short RNA primers. DNA polymerase synthesizes DNA in the 5′ to 3′ direction because the enzyme requires a free 3′-hydroxyl. Since the two DNA strands are antiparallel, the continuous addition of deoxyribonucleotides to the RNA primer can occur on only one strand (Fig. 5-2). The DNA strand replicated continuously is called the *leading strand*. On the opposite strand, called the *lagging strand*, deoxyribonucleotides are added discontinuously, that is, short pieces of DNA (Okazaki fragments) are synthesized on many RNA primers that are points of initiation. Later the RNA primers are removed and the regions that are left vacant are filled with deoxyribonucleotides. Simultaneous synthesis of antiparallel strands is called *bidirectional synthesis* (some viruses replicate in a unidirectional manner—only one strand is synthesized at a time). The smaller units of DNA synthesized on the lagging strand are united by the enzyme called *DNA ligase*.

Fig. 5-1. Representation of the Meselson and Stahl experiment demonstrating semiconservative replication. Microbial cells are grown in a medium containing heavy nitrogen (N^{15}). A. Both strands of DNA are labeled with heavy isotope. B. After transfer to a medium containing light nitrogen (N^{14}) this generation of cells contains DNA that has one light and one heavy strand. C. One-half of the progeny in this generation of cells contain hybrid DNA ($N^{15}N^{14}$). D. One-fourth of the progeny contain hybrid DNA, while three-fourths contain light DNA. (From R. F. Boyd and J. J. Marr (Eds.). *Medical Microbiology*. Boston: Little, Brown, 1980.)

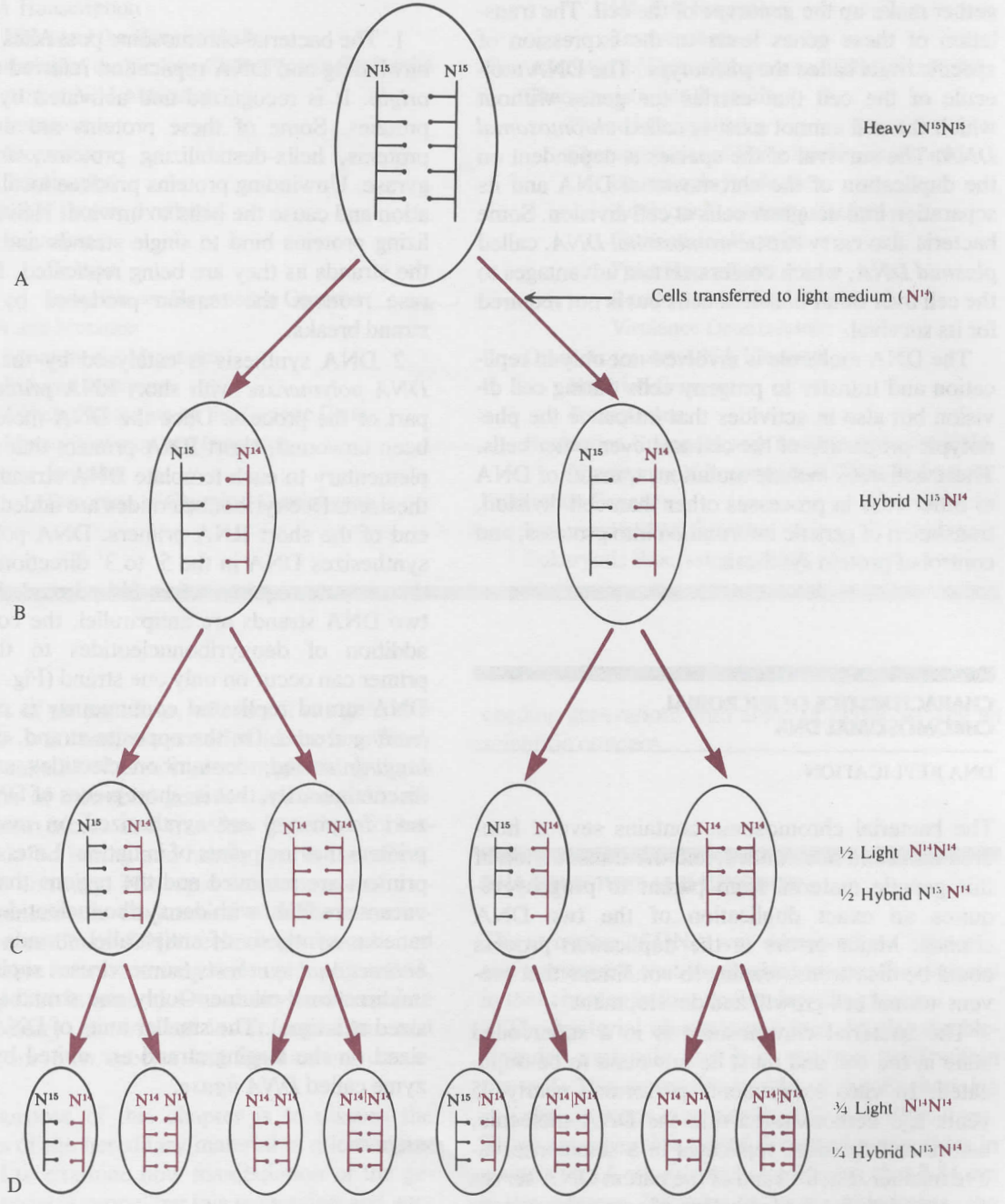


Fig. 5-2. Proposed mechanism for replication of DNA in vivo. The circles represent various proteins or enzymes that are involved in the unwinding and stabilization of the DNA strands. A. Helical unwinding of DNA during replication. B. Opposite polarity of DNA synthesis. On the lagging strand DNA synthesis is discontinuous and each DNA fragment is first primed by ribonucleotides. Later the fragments are sealed by DNA ligase. (● = unwinding proteins, which initiate localized denaturation of DNA helix and cause it to unwind; ○ = destabilizing proteins, which bind to single strands of DNA that were unwound by unwinding proteins and stabilize them; ● = gyrase, which relaxes the tension produced on the helix when the DNA is unwound; ••• = ribonucleotides used as primer for the fragments of DNA produced on the lagging strand in particular.)

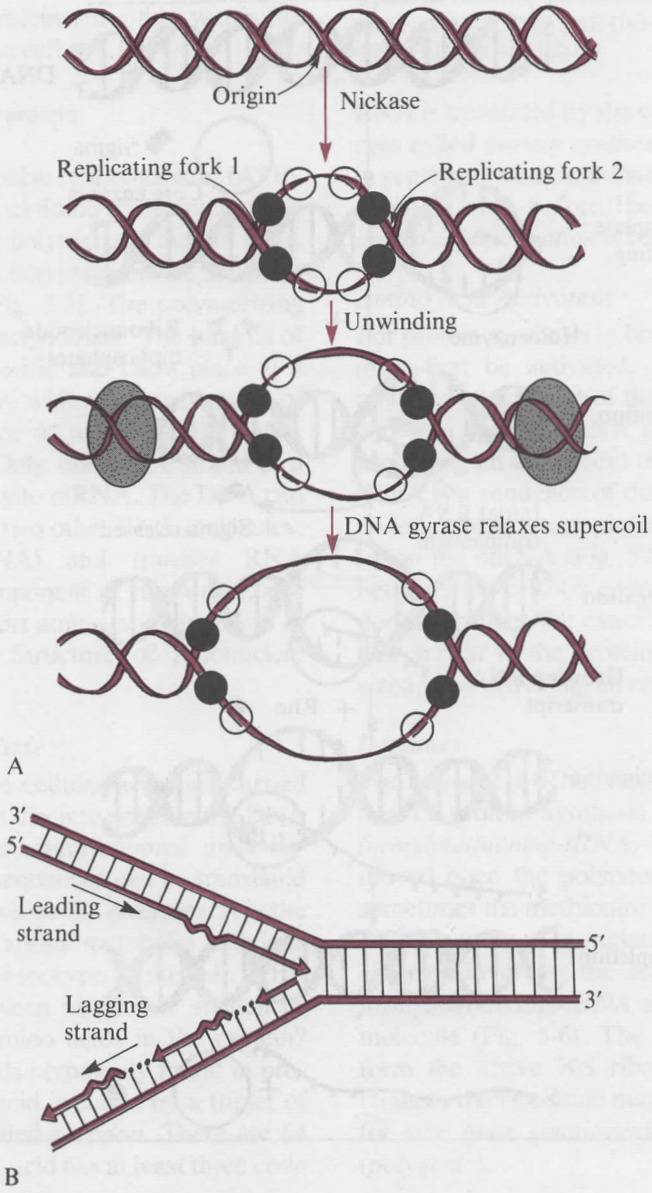
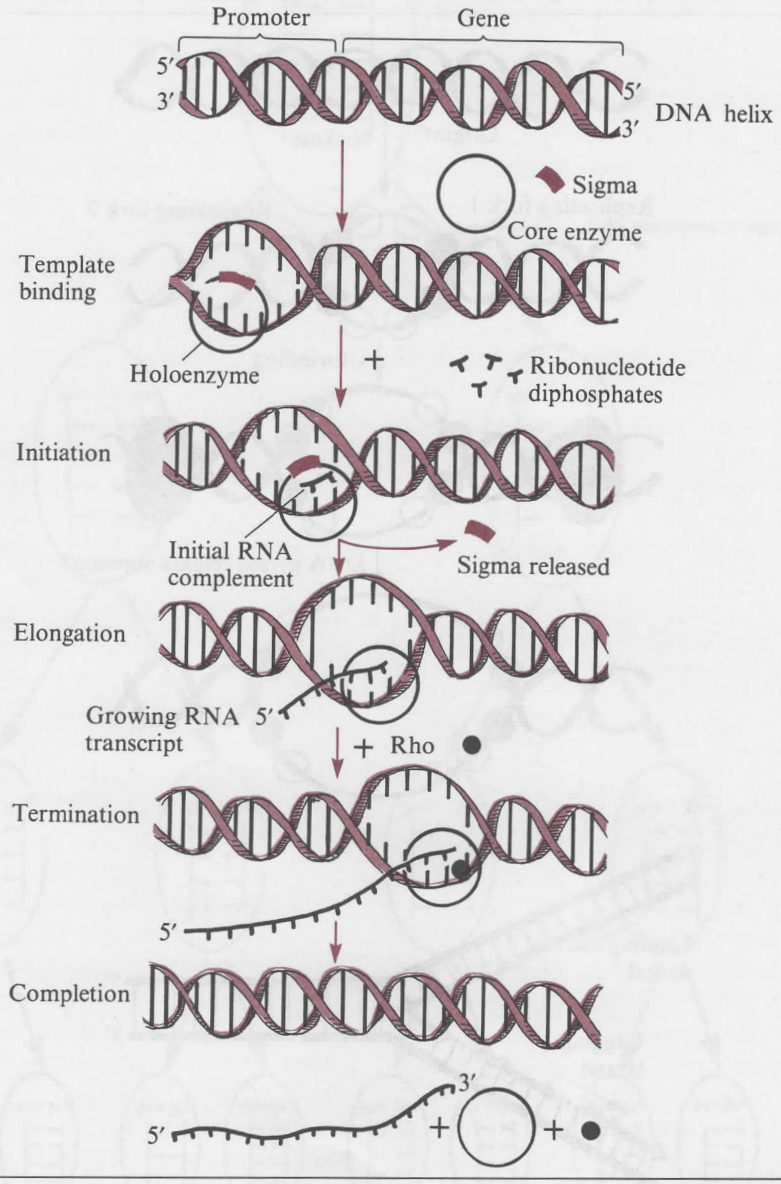


Fig. 5-3. Transcription of DNA. DNA is transcribed by an RNA polymerase that consists of a core plus a subunit called *sigma factor*. The polymerase transcribes a single DNA strand at an initiation site (also called *promoter site*). The sigma factor is required at initiation; once transcription has been initiated the sigma factor is released. The mRNA transcript continues to be synthesized until a termination site on the DNA is reached. The termination site is recognized by a protein called *rho*. The mRNA is released, and core enzyme and sigma factor will bind to be used again to initiate a new mRNA transcript. (Adapted from W. S. Klug and M. R. Cummings, *Concepts of Genetics*. Columbus, OH: Merrill, 1983.)



DNA TRANSCRIPTION

The information coded on the DNA molecule can be transcribed on a complementary molecule called messenger RNA (mRNA). RNA has a ribose sugar instead of deoxyribose and the base uracil is used in place of thymine. It is the information in the mRNA molecule that is translated into amino acid sequences and hence proteins. In other words the flow of information in the cell is

DNA ⟶ RNA ⟶ protein

The nucleotides adenosine triphosphate (ATP), guanosine triphosphate, cytidine triphosphate, and uridine triphosphate are polymerized into an RNA chain in the 5′ to 3′ direction using one of the DNA chains as a template (Fig. 5-3). The polymerizing enzyme is called *RNA polymerase*. The binding of RNA polymerase is specific and takes place at a site called the *promoter*, while the termination of transcription takes place at a site on the DNA called the *terminator*. Only one DNA strand at a time can be transcribed into mRNA. The DNA can also be transcribed into two other RNA molecules, ribosomal RNA (rRNA) and transfer RNA (tRNA). rRNA is a component of ribosomes, and tRNA is used to transport amino acids involved in protein synthesis (see Structure of Ribonucleic Acid, Chapter 2).

DNA and the Genetic Code

The information for the cellular activities carried out by the cell resides in discrete units on the DNA called genes. Genes are informational units because their nucleotide sequences can be translated into proteins, many of which are enzymes. It is the enzymes involved in various metabolic reactions that contribute to the phenotype of the cell. What is the relationship between nucleotide sequences and the sequence of amino acids in the protein? There are 20 amino acids commonly found in proteins, and each amino acid is coded by a triplet of bases in the mRNA called a *codon*. There are 64 codons, and each amino acid has at least three code words (Table 5-1); in other words, the code is *degenerate*. Codons that specify amino acids are referred to as *sense codons*. Three of the codons do not code for an amino acid and are referred to as *nonsense codons*. The function of nonsense codons will be discussed shortly.

TRANSLATION OF THE GENETIC CODE (PROTEIN SYNTHESIS)

RNA is translated by the cell into proteins in a process called *protein synthesis*. There are three steps in protein synthesis: *initiation, elongation*, and *termination*, but before these occur a step called *amino acid activation* is required.

Amino Acid Activation

For protein synthesis to be initiated the amino acids must first be activated. Activation involves the covalent attachment of the amino acid to the CCA end of a specific tRNA molecule (Fig. 5-4), thus producing an aminoacyl tRNA. At one site on the tRNA is a sequence of three bases called the *anticodon*, which is complementary to the codon triplet on the mRNA (Fig. 5-5). The complementarity between the mRNA codon and the tRNA anticodon determines the exact sequence of amino acids that appear in the protein molecule to be synthesized, thus providing an errorless translation.

Initiation

The aminoacyl tRNA that is required for the initiation of protein synthesis in bacterial cells is *N-formylmethionine-tRNA*. The formyl group is removed once the polypeptide is synthesized, and sometimes the methionine is also removed.

The next step in initiation is the formation of a complex involving the 30S ribosomal subunit, *N-formylmethionine-tRNA* and the messenger RNA molecule (Fig. 5-6). The 50S subunit adds on to form the active 70S ribosome. The mRNA that binds to the ribosome may contain the information for one gene (*monogenic*) or for several genes (*polygenic*).

Table 5-1. The Genetic Code

First Letter	Second Letter: U	C	A	G	Third Letter
U	Phe	Ser	Tyr	Cys	U
	Phe	Ser	Tyr	Cys	C
	Leu	Ser	Nonsense	Nonsense	A
	Leu	Ser	Nonsense	Trp	G
C	Leu	Pro	His	Arg	U
	Leu	Pro	His	Arg	C
	Leu	Pro	Gln	Arg	A
	Leu	Pro	Gln	Arg	G
A	Ileu	Thr	Asn	Ser	U
	Ileu	Thr	Asn	Ser	C
	Ileu	Thr	Lys	Arg	A
	Met	Thr	Lys	Arg	G
G	Val	Ala	Asp	Gly	U
	Val	Ala	Asp	Gly	C
	Val	Ala	Glu	Gly	A
	Val	Ala	Glu	Gly	G

U = uracil; C = cytosine; A = adenine; G = guanine; Phe = phenylalanine; Ser = serine; Tyr = tyrosine; Cys = cysteine; Leu = leucine; Trp = tryptophan; Pro = proline; His = histidine; Arg = arginine; Gln = glutamine; Ileu = isoleucine; Thr = threonine; Asn = asparagine; Lys = lysine; Met = methionine; Val = valine; Ala = alanine; Asp = aspartic acid; Gly = glycine; Glu = glutamic acid.

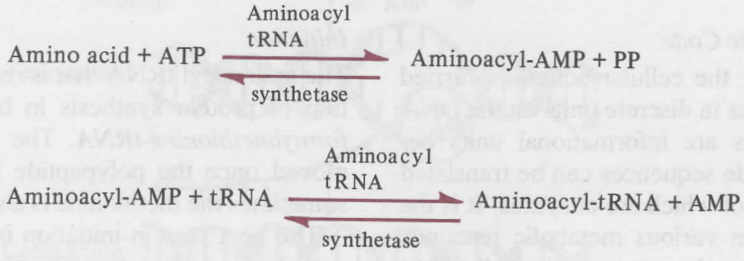
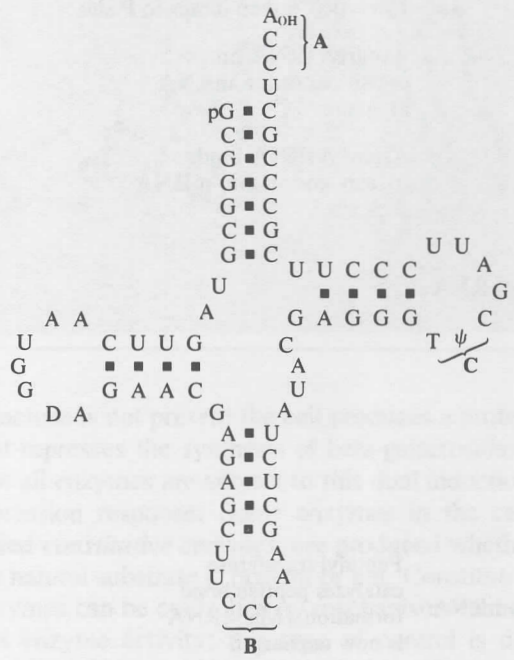


Fig. 5-4. Reactions involved in the activation of amino acids and their attachment to the CCA end of specific tRNA molecules. (AMP = adenosine monophosphate; PP = pyrophosphate.)

Fig. 5-5. Structure of glycyl tRNA. The CCA end (*A*) is common to all transfer RNA's. The 3′-OH group of the terminal adenine serves as the site of an ester linkage to the carboxyl group of glycine. The CCC triplet (*B*) is the anticodon site for attachment to the codon GGG. The triplet TψC (*C*) may be the site for attachment of the tRNA to the ribosome. (A = adenine; C = cytosine; U = uracil; G = guanine; T = thymine; D = 5,6-dihydrouracil; ψ = pseudouracil.)



Elongation

The 70S ribosome has two sites for binding aminoacyl tRNAs. One site, called the *A* (*acceptor*) *site*, binds new aminoacyl tRNA molecules; the other, the *P* (*peptide*) *site* represents the site of the growing polypeptide chain. What happens initially is that N-formylmethionine-tRNA binds to the P site. (It is not known if N-formylmethionine-tRNA enters the A site and then moves to the P site or binds directly to the P site.) The next mRNA codon is now opposite the A site, and once this site is occupied a peptide bond is formed with the aid of the enzyme peptidyl transferase, which is found on the 50S ribosome. Once the peptide is formed the ribosome moves along the mRNA by one codon—a process that places the peptidyl tRNA on the P site and frees the A site (Fig. 5-6). The A site is now ready to accept the next aminoacyl tRNA whose anticodon is complementary to the mRNA codon. As the ribosome moves down the mRNA chain the initial ribosomal binding site is freed and a second ribosome attaches and begins protein synthesis. The association of a number of ribosomes in various stages of protein synthesis (Fig. 5-7) forms a *polyribosome* or *polysome*.

Termination

Termination of protein synthesis on the mRNA chain is indicated by a special codon called a *nonsense codon*. Three such codons are UAA, UAG, and UGA. Termination involves cleavage of the terminal end of the polypeptide chain from the tRNA bound to the P site on the ribosome. This ribosome will either dissociate from monogenic mRNA or move along to another initiation sequence if the mRNA is polygenic.

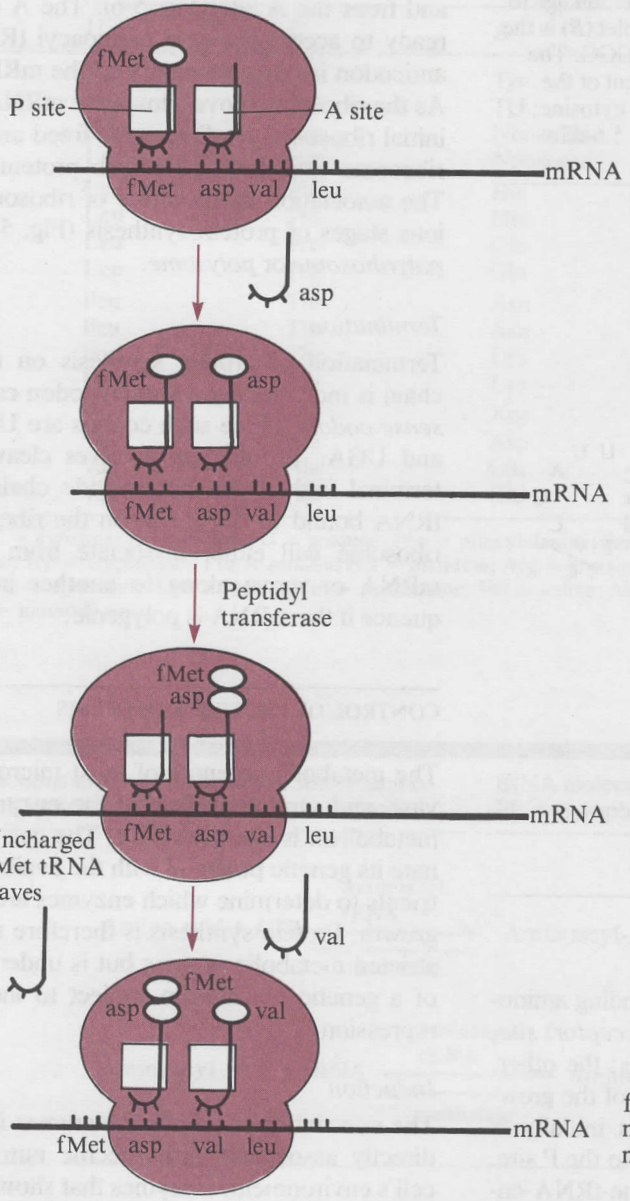
CONTROL OF PROTEIN SYNTHESIS

The metabolic potential of most microbial cells is vast, and synthesis of all of the enzymes used in metabolism is not necessary. The cell will coordinate its genetic potential with the availability of nutrients to determine which enzymes are needed for growth. Protein synthesis is therefore not an unrestricted metabolic process but is under the control of a genetic mechanism subject to induction and repression.

Induction

The concentration of many enzymes in the cell is directly associated with specific nutrients in the cell's environment. Enzymes that show an increase in concentration in the presence of specific nutrients are called *inducible* enzymes. The enzyme beta-galactosidase, for example, is an inducible enzyme that is produced when its substrate, lactose, a disaccharide, is present in the cell's environment.

Fig. 5-6. Mechanism of protein synthesis. See text for further discussion. (fmet = formylmethionine; asp = aspartic acid; val = valine; leu = leucine.)



Formation of 70S complex and binding of fMethionyL tRNA to A site. fMet-tRNA then jumps to P site.

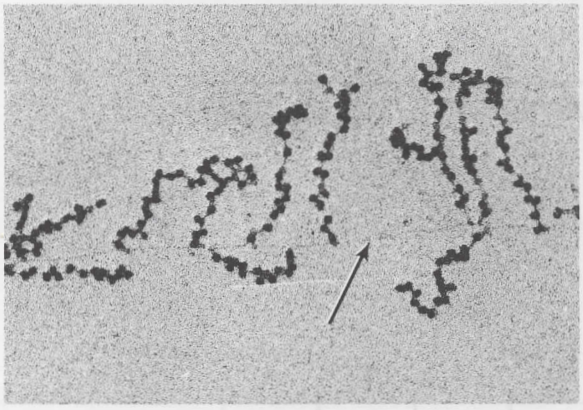
Aspartyl tRNA binds to asp codon on mRNA at A site.

Aspartyl tRNA binds to asp codon on mRNA at A site.

Peptidyl transferase catalyzes peptide bond formation. fMet-tRNA is now uncharged and returns to cytoplasm.

fMet-asp peptide moves to P site. A site now ready to accept valyl tRNA.

Fig. 5-7. Polysomes. The horizontal fiber (*arrow*) is the single strand of DNA to which are attached mRNA molecules in various stages of translation. To each mRNA are attached ribosomes (the dark granules), which increase in number from left to right (the direction of translation). (From O. L. Miller, Jr., *Cold Spring Harbor Symp. Quant. Biol.* 35:505, 1970.)



If lactose is not present the cell produces a protein that represses the synthesis of beta-galactosidase. Not all enzymes are subject to this dual induction-repression response. Some enzymes in the cell, called *constitutive enzymes*, are produced whether the natural substrate is present or not. Constitutive enzymes can be controlled by mechanisms that affect enzyme activity; this type of control is discussed in Chapter 9. Much of our knowledge concerning induction and repression is the result of work by two French microbiologists, Jacob and Monod. They were instrumental in developing the operon theory of control.

OPERON. The operon consists of a cluster of genes and other sites whose presence is associated with the control of a translation process involving a single metabolic product. For example, when the bacterial cell synthesizes an amino acid or catabolizes a carbohydrate several enzymes are usually involved. These enzymes are coded by genes, called *structural genes*, that are clustered together in the operon. The most widely studied repression-induction system is the lactose operon (lac operon), which has the following components (Fig. 5-8): The structural genes of the lactose operon are the beta-galactosidase gene (Z gene), the permease gene (Y gene), and the transacetylase gene (A gene). Beta-galactosidase cleaves lactose into glucose and galactose. The permease gene is responsible for transporting lactose across the cytoplasmic membrane. The exact function of the transacetylase gene has not been completely elucidated.

The *operator* is a sequence of nucleotides that lies adjacent to the structural genes and is involved in their control. In the absence of lactose in the medium, the cell has no need to produce lactose-catabolizing enzymes and the transcription of the structural genes is therefore inhibited (Fig. 5-8). This inhibition is due to the binding of a repressor protein to the operator, which prevents transcription of the adjacent structural genes.

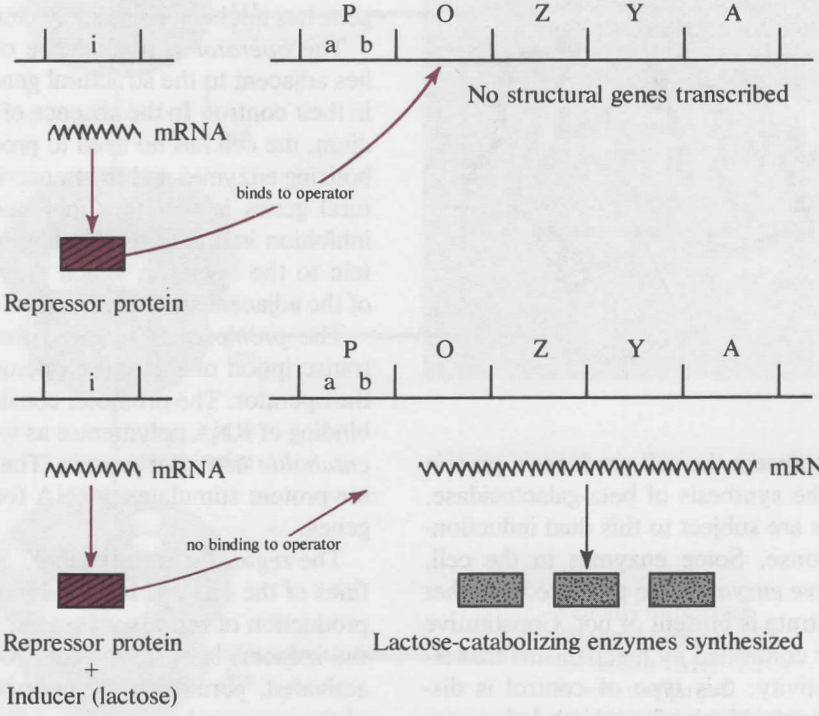
The *promoter* is required for the initiation of transcription of the entire operon and lies outside the operator. The promoter consists of a site for the binding of RNA polymerase as well as a site for the *catabolite activator protein*. The catabolite activator protein stimulates mRNA formation of the lac genes.

The *regulator* gene (i gene) lies outside the confines of the operon. It contains information for the production of repressor protein. In the presence of the inducer, lactose, the repressor molecule is inactivated, permitting the unimpeded transcription of the structural genes (Fig. 5-8). In the absence of lactose, the repressor molecule binds to the operator and prevents the transcription of the structural genes by RNA polymerase.

Inducible vs. Repressible Operons

The lactose operon is an example of an inducible system in which the operon controls the breakdown of a compound (lactose). There are operons, called *repressible operons*, that control the synthesis of a compound, for example, the tryptophan operon, which controls the synthesis of the amino acid tryptophan. Repressible operons have a regulatory gene that codes for an *aporepressor* protein that cannot bind to the operator unless it is first complexed with a *corepressor*, which in this in-

Fig. 5-8. Operon model. Repression and induction on the lactose (lac) operon. The operon consists of promoter (*P*) region that contains sites for binding of RNA polymerase (*a*) and catabolite activator protein (*b*). Operator (*O*) controls transcription of structural genes Z (beta-galactosidase), Y (permease), and A (transacetylase). *i* is the unlinked regulatory gene. A. The lac operon is repressed. Repressor protein is synthesized and binds to operator, preventing transcription of structural genes. B. Induction of lac operon. Inducer lactose binds to repressor protein and prevents binding to operator. Structural genes are transcribed, and lactose-catabolizing enzymes are synthesized.

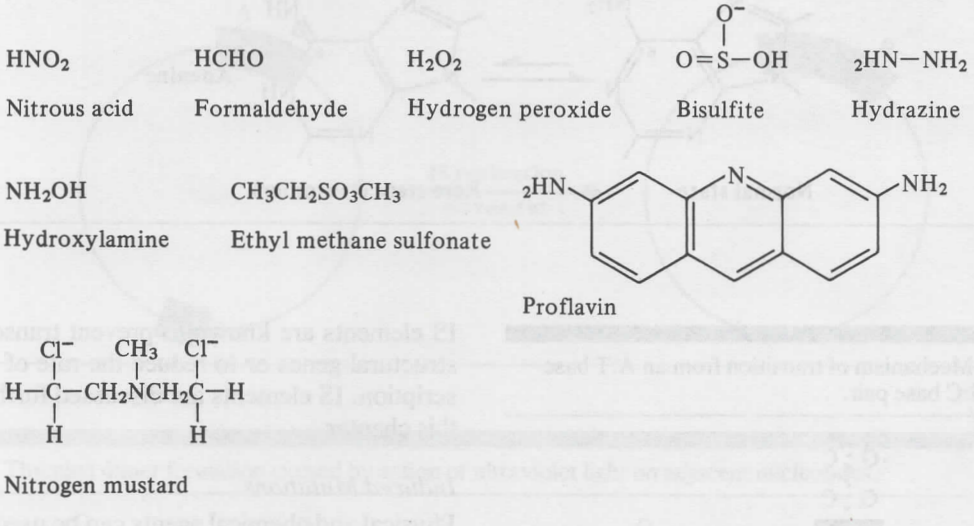


stance is the amino acid tryptophan or a derivative of it such as tryptophan-tRNA. The presence of excess tryptophan in a culture medium results in the formation of a functional repressor that binds to the tryptophan operon and prevents the synthesis of enzymes involved in the synthesis of tryptophan.

DNA AND MUTATION

Microorganisms like other biological systems must replicate their DNA with a high degree of fidelity to maintain their species identity. Some errors are needed in the genetic material, however, for the species also to evolve. Changes in the genotype or base sequence of the hereditary material are referred to as *mutations*. Mutations may bring about advantages or disadvantages to the cell. Some mutations may be lethal, while others may help the organism to survive in its environment. The induction of mutations is called *mutagenesis*, and the agent involved is called a *mutagen*. Mutations play an important role in many fields of microbiology. They are used by research scientists to delve into the various aspects of structure and function, metabolic processes, and reproduction of microorganisms. The results from such studies have been helpful in

Fig. 5-9. Structure of some chemical mutagens.



understanding the genetic systems of higher organisms. Mutation is also important in applied areas of microbiology. Increased synthesis of various industrially used products resulting from microbial metabolism is due to the ability of microbiologists to select naturally occurring mutants or to induce mutation.

Mutations may arise in two general ways: They may arise *spontaneously* or they may be *induced*. The probability of a mutation arising spontaneously may be only one in a million; that is, the mutation rate is 1×10^{-6} or even greater, for example, 1×10^{-4}. The frequency at which mutations may arise can be increased substantially by inducing them with a *mutagenic agent*. Mutagens include physical agents such as ultraviolet light and x-rays as well as chemical agents such as nitrous acid, hydrogen peroxide, nitrogen mustard, and others (Fig. 5-9).

Spontaneous Mutations

Spontaneous mutations are those mutations that arise without the intervention of a mutagen. For example, the purine and pyrimidine bases on the DNA molecule can exist in two electrochemical forms called *tautomers*. In the case of adenine (Fig. 5-10) the stable form of the base possesses a NH_2 group at position 6, but in its rare form one of the hydrogens of NH_2 moves to position 1. Adenine, which normally pairs with guanine, can now bond with cytosine. This type of mutation results in a change in the base pairing of progeny DNA molecules and is referred to as a *transition* (Fig. 5-11). In other words what was originally an A:T base pair in the parent molecule becomes a G:C base pair in the progeny. A transition represents the substitution of one purine for another purine, but it can also involve the substitution of one pyrimidine for another.

Spontaneous mutations may also occur by a biological mechanism. It has been observed that specific nucleotide sequences or elements called *insertion sequences* (IS) are present in the DNA molecule. IS are usually less than 2000 nucleotide sequences in length and are capable of moving from one location on the DNA molecule to another. IS elements do not confer any known phenotype on the cell, but they can insert themselves within and between genes and thus affect the activity of genes at the site of the insertion or distal to it (Fig. 5-12).

Fig. 5-10. Adenine in its normal and rare state.

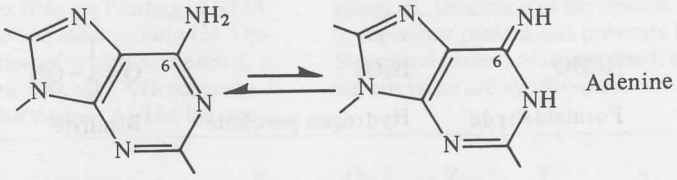
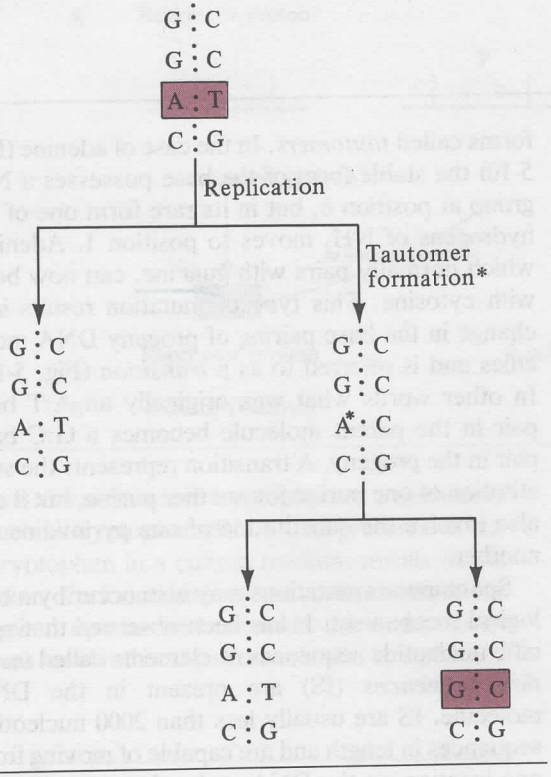


Fig. 5-11. Mechanism of transition from an A:T base pair to a G:C base pair.



IS elements are known to prevent transcription of structural genes or to reduce the rate of their transcription. IS elements are discussed further later in this chapter.

Induced Mutations

Physical and chemical agents can be used to induce mutations in the DNA. X-rays or gamma rays can break DNA strands, which may result in chromosomal rearrangement or may lead to the death of the cell. Ionizing radiations interact with molecules in the cell, such as water, to produce free radicals (·OH) with tremendous oxidizing powers that cause breaks in the DNA molecule (leading to deletions). Ultraviolet light is a frequently used mutagenic agent that causes pyrimidine dimer formation (Fig. 5-13). Pyrimidine dimers cause distortions in the DNA molecule, and mutation arises when the bacterial cell repairs the mutation but does it incorrectly. The distortions imposed on the DNA molecule by pyrimidine dimer formation can be repaired through the induction of a number of enzymes. The dimer can be deleted by two major processes: *photoreactivation*, which occurs only in the presence of light, and *dark repair*, which occurs only in the absence of light. Photoreactivation occurs by an enzymatic splitting of the bonds forming the dimer, which results in regeneration of the original pyrimidine (Fig. 5-14A). Dark repair requires several enzymes induced by ultraviolet light. The basis of repair is that the pyrimidine dimer is removed along with adjacent nucleotides. A polymerase catalyzes

Fig. 5-12. Movement of an insertion sequence (*IS*) from one site on the DNA molecule to a site between two genes (*A* and *B*) on the same DNA molecule. Actually, a copy of the IS element inserts between genes A and B.

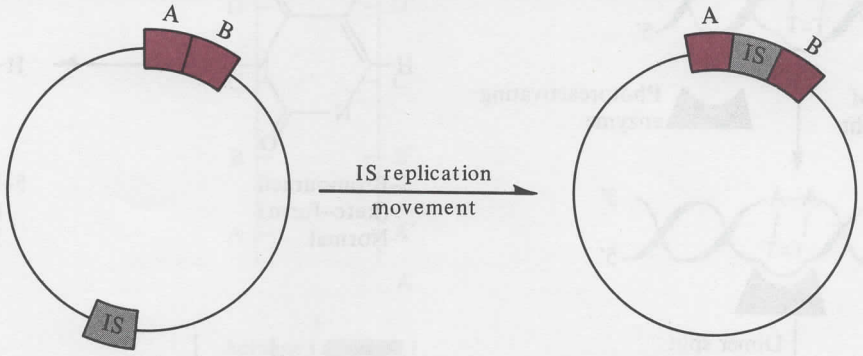
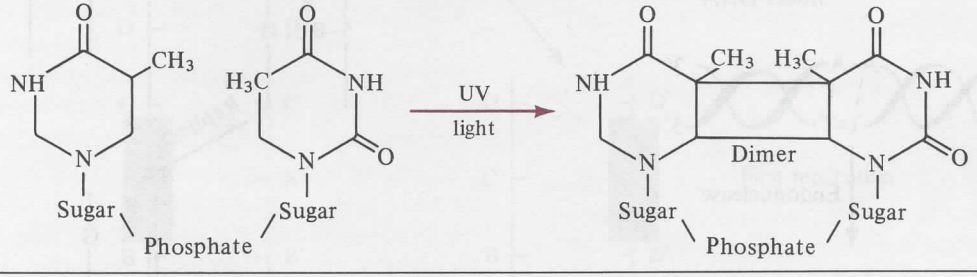


Fig. 5-13. Thymine dimer formation caused by action of ultraviolet light on adjacent nucleotides.



the resynthesis of the excised pyrimidines plus the adjacent nucleotides (Fig. 5-14B). A third type of repair mechanism called *postreplication repair* occurs in those cells lacking the enzymes for light and dark repair. Postreplication repair is believed to be error prone resulting in mutations.

Chemical mutagens can produce different types of mutation. Base analogues, such as 5-bromouracil (5-BU) and 2-aminopurine, that are structurally similar but not identical to the normal DNA base can cause transitions. 5-BU is an analogue of thymine and can exist in two different states. In its normal state 5-BU pairs with adenine, but in its rare state it pairs with guanine (Fig. 5-15).

Hydroxylamine (NH_2OH), nitrous acid (HNO_2), and bisulfite (HSO_3) are deaminating agents that remove the amino group from purine and pyrimidine bases. Cytosine, for example, can be deaminated to uracil (Fig. 5-16). This leads to a transition; that is, the deaminated cytosine will pair with adenine instead of guanine. The adenine of the progeny strand will pair with thymine.

Chemicals such as acridines are capable of inserting themselves (intercalation) between bases on the DNA. This results in distortion of the DNA and can result in insertions or deletion of single base pairs (Fig. 5-17).

Chemicals such as nitrogen mustard (Fig. 5-18) interact with positions 1 and 7 of guanine, bringing about their alkylation. Alkylation causes hydrolysis of the purine (depurination) and formation of a gap in the DNA molecule.

Fig. 5-14. DNA repair mechanisms following thymine dimer formation. A. Photoreactivation repair. B. Dark repair. (Adapted from E. M. Witkin, *Bacteriol. Rev.* 40:869, 1976.)

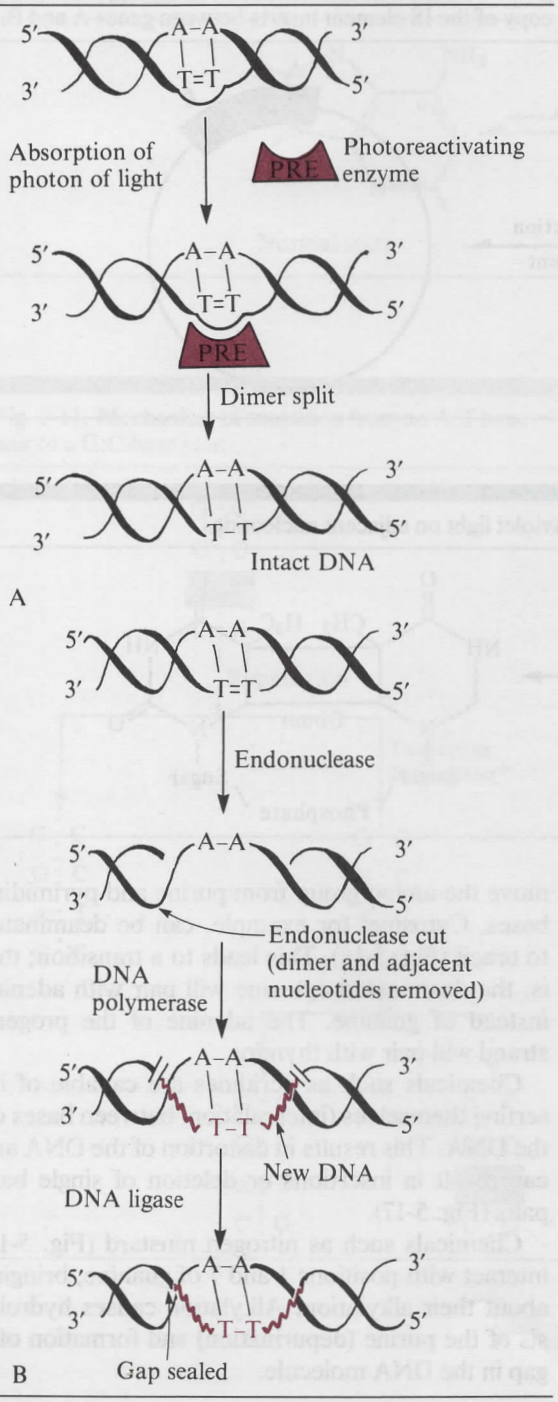


Fig. 5-15. A. Conversion of 5-bromouracil (5-BU), a mutagen, from its normal keto-form into the more rare enol-form. B. Mechanism by which 5-BU causes transitions. (5-BU is rare enol-form.)

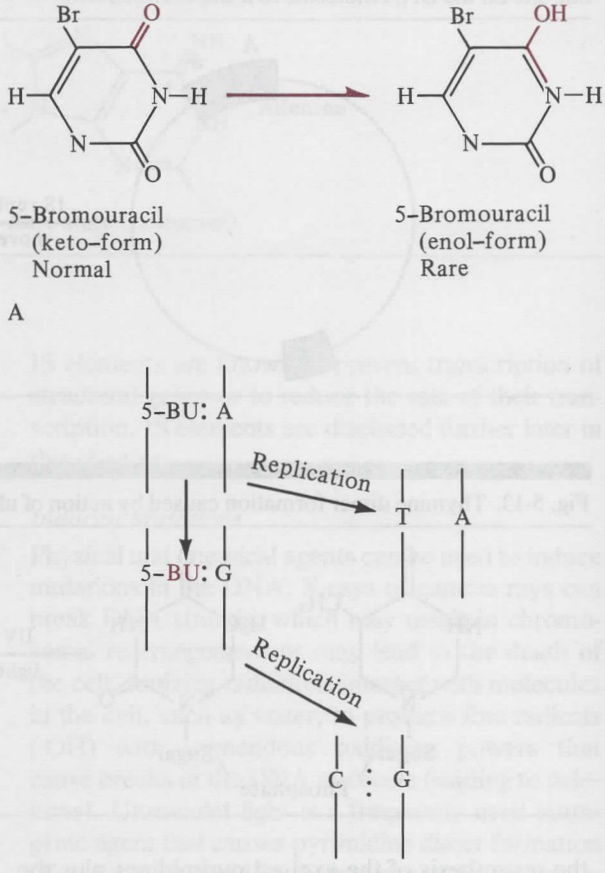


Fig. 5-16. Deamination of cytosine to uracil.

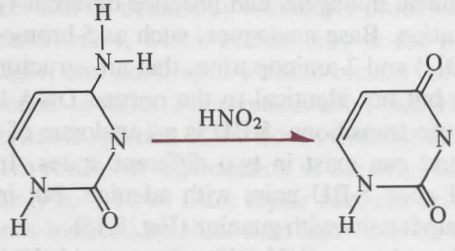


Fig. 5-17. Acridine insertion into DNA. Acridine can be inserted into a template strand of DNA (*left*). Replication results in the addition of a base (*X′*). If the acridine is inserted into the daughter strand (*right*), a base (*C*) will be deleted after replication. (From W. Hayes, *The Genetics of Bacteria and Their Viruses* [2nd ed.]. Oxford, England: Blackwell, 1968.)

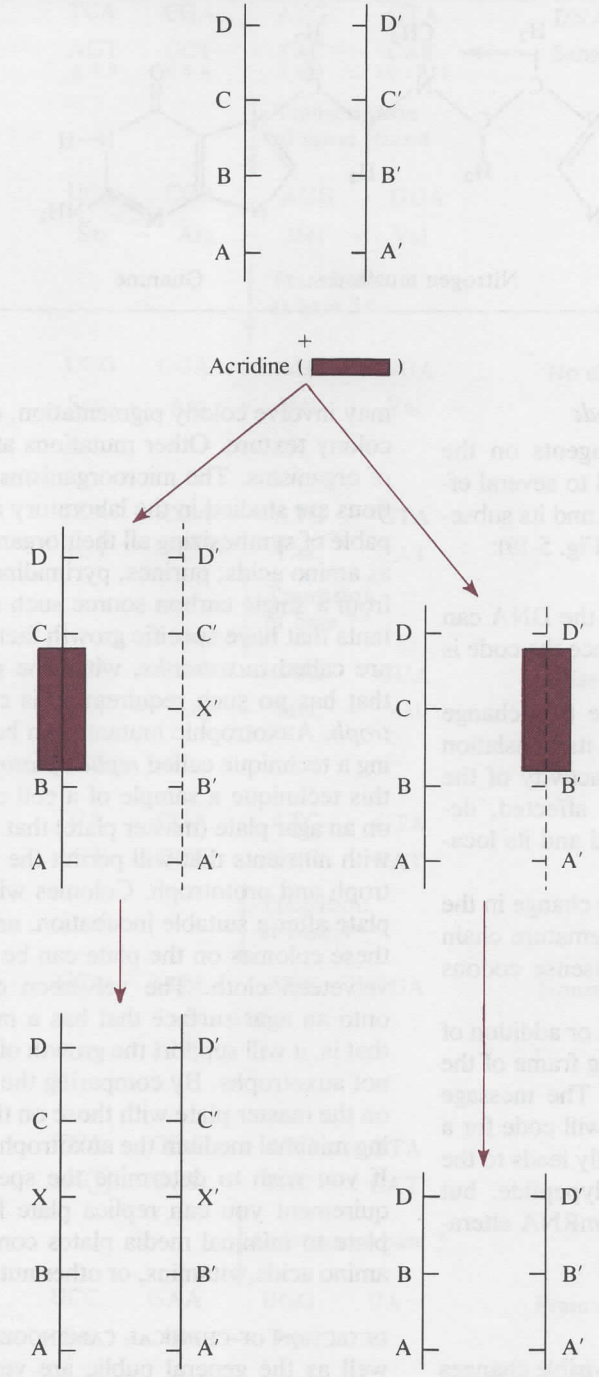
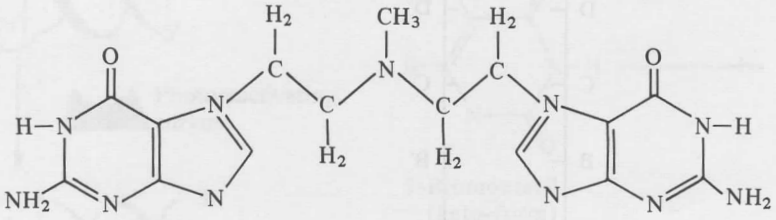


Fig. 5-18. Alkylation of adjacent guanine residues in DNA by nitrogen mustard.



Effect of Mutation on the Genetic Code

The various effects of mutagenic agents on the bases in the DNA molecule can lead to several effects on the transcription of the gene and its subsequent translation into a polypeptide (Fig. 5-19):

1. No effect. Alteration of a base in the DNA can lead to no changes in the codon since the code is degenerate.
2. Missense mutation. A single base pair change leads to a change in the codon and its translation into a different amino acid. The activity of the polypeptide may or may not be affected, depending on the type of amino acid and its location in the peptide chain.
3. Nonsense mutation. A single base change in the DNA can occasionally lead to premature chain termination on the mRNA if nonsense codons are formed.
4. Frameshift mutation. The deletion or addition of a single base can cause the reading frame of the message to be shifted one base. The message distal to the insertion or deletion will code for a new set of amino acids. This usually leads to the formation of a nonfunctional polypeptide, but again this depends on the site of mRNA alteration.

Mutant Isolation and Detection

ISOLATION. Some mutations result in visible changes that are easily detected. In bacteria these changes may involve colony pigmentation, colony size, and colony texture. Other mutations affect the growth of organisms. The microorganisms in which mutations are studied in the laboratory are normally capable of synthesizing all their organic material such as amino acids, purines, pyrimidines, and vitamins from a single carbon source such as glucose. Mutants that have specific growth factor requirements are called *auxotrophs*, while the parent organism that has no such requirement is called the *prototroph*. Auxotrophic mutants can be isolated by using a technique called *replica plating* (Fig. 5-20). In this technique a sample of a cell culture is spread on an agar plate (master plate) that is supplemented with nutrients that will permit the growth of auxotroph and prototroph. Colonies will appear on the plate after a suitable incubation, and the pattern of these colonies on the plate can be transferred to a velveteen cloth. The velveteen cloth is pressed onto an agar surface that has a minimal medium, that is, it will support the growth of prototrophs but not auxotrophs. By comparing the colony patterns on the master plate with those on the plate containing minimal medium the auxotroph can be isolated. If you wish to determine the specific mutant requirement you can replica plate from the master plate to minimal media plates containing different amino acids, vitamins, or other nutrients.

DETECTION OF CHEMICAL CARCINOGENS. Scientists as well as the general public are very aware of the need to detect chemicals that can cause cancer

Fig. 5-19. Effects of mutation on the genetic code. A. Genetic change that produces no change in the peptide. B. Mutation that causes missense or change in the amino acid. C. Mutation that results in the formation of a nonsense or terminating codon. D. Mutation in which a base is deleted that results in a frameshift or change in all the amino acids distal to the initial mutation. (Ser = serine; Arg = arginine; Met = methionine; Val = valine; Glu = glutamic acid; Trp = tryptophan.)

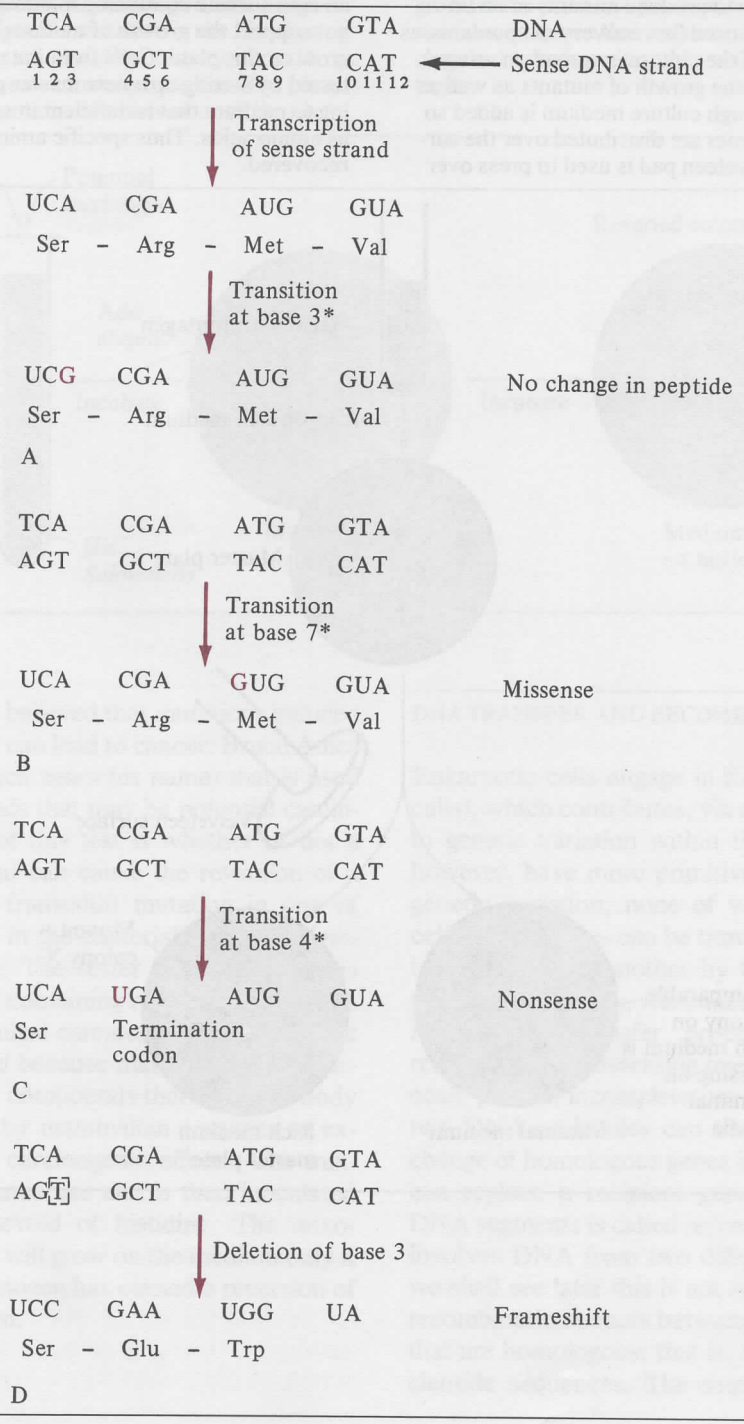


Fig. 5-20. Replica plating technique. A culture may be treated with a mutagen to produce mutants or an untreated culture may be used for recovery of spontaneous mutants. An aliquot of the culture is spread on an agar medium that supports the growth of mutants as well as nonmutants. Only enough culture medium is added so that less than 100 colonies are distributed over the surface of the agar. A velveteen pad is used to press over the colonies (master plate), and they are transferred to an agar surface containing minimal medium (which will not support the growth of mutants). Colony X does not grow on this plate. Cells from the mutant colony can be tested by setting up a new master plate and replica plating to medium that is deficient in specific nutrients such as amino acids. Thus specific amino acid mutants can be recovered.

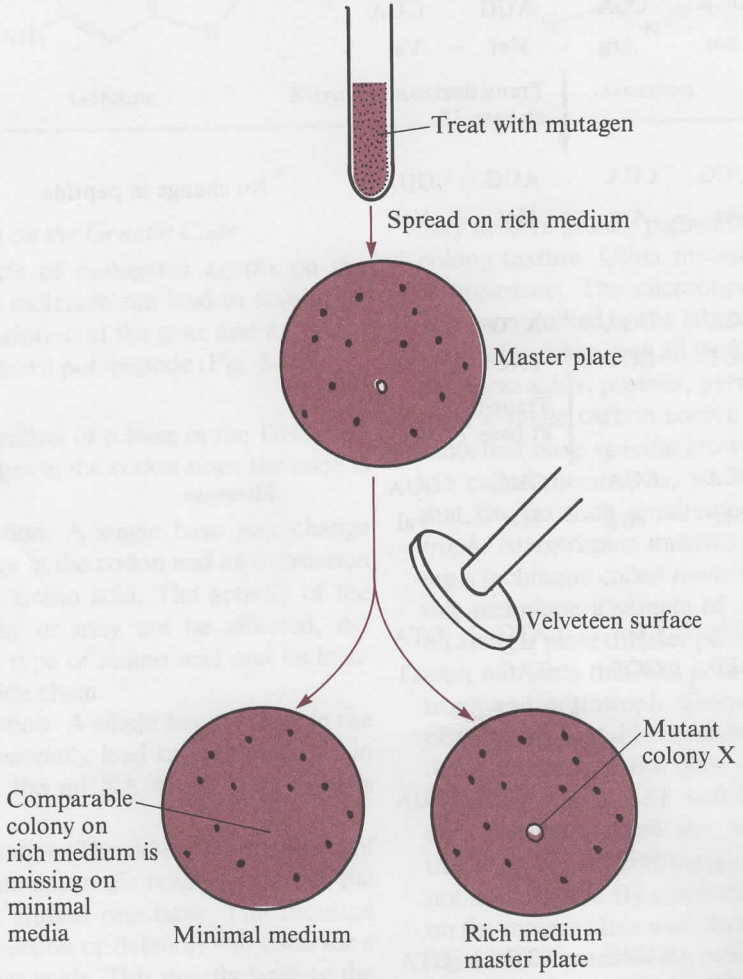
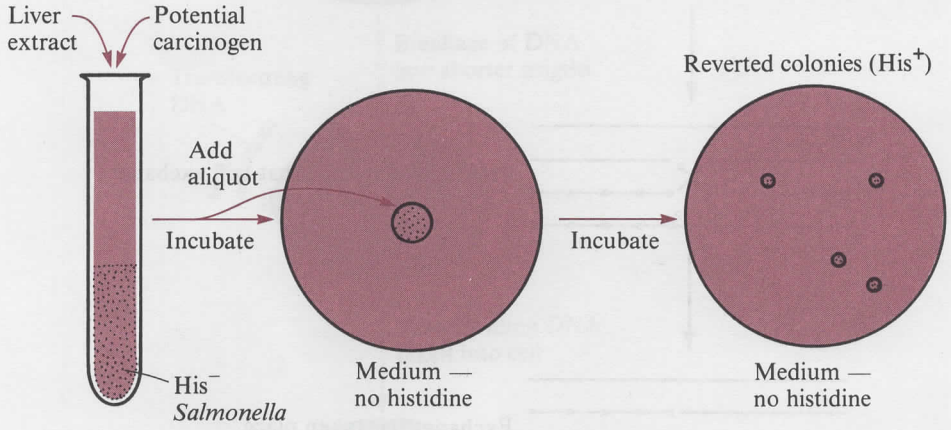


Fig. 5-21. Ames test for carcinogenicity. A mixture containing liver extract, potential carcinogen, and *Salmonella* species with a specific mutation in the histidine operon (*his*$^-$) are incubated. An aliquot is transferred to an agar medium that does not contain histidine. The plate is incubated, and any bacteria that have reverted to his$^+$ because of the mutagenic effect of the carcinogen will grow on the plate and produce colonies. Liver extract contains enzymes that are normally involved in the biochemical transformation of a chemical to an active mutagen.

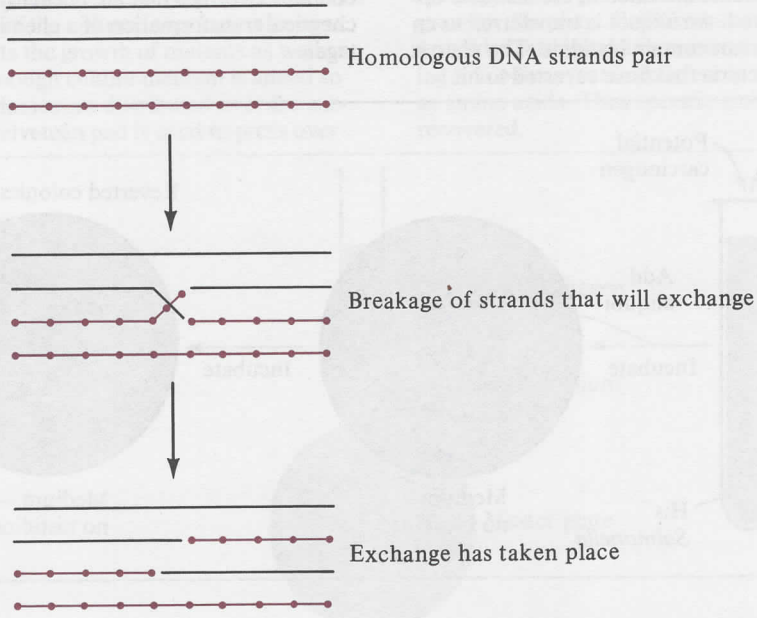


(*carcinogens*). It is believed that mutations induced by chemical agents can lead to cancer. Bruce Ames devised a test (which bears his name) that is used to screen compounds that may be potential carcinogens. The basis of this test is whether or not a chemical compound can cause the reversion of a well-characterized frameshift mutation in one of the histidine genes in the bacterial species *Salmonella typhimurium*. The tester bacterial strain is added to a mixture containing rat liver extract plus the suspected chemical carcinogen (Fig. 5-21). Rat liver extract is used because many potentially carcinogenic chemical compounds that enter the body must be activated by mammalian enzymes to exhibit mutagenic or carcinogenic effects. The mixture is allowed to incubate and is then inoculated onto a medium devoid of histidine. The auxotrophic *Salmonella* will grow on the medium only if the suspected carcinogen has caused a reversion of the original mutation.

DNA TRANSFER AND RECOMBINATION

Eukaryotic cells engage in fusion of gametes (sex cells), which contributes, via chromosome meiosis, to genetic variation within the species. Bacteria, however, have more primitive means of acquiring genetic variation, none of which involve natural cell fusion. Genes can be transferred from one bacterial species to another by three general mechanisms: *conjugation, transduction*, and *transformation*. These transfer processes result in the recipient cell's possessing one complete DNA molecule plus an incomplete one from the donor. The two DNA molecules can then engage in the exchange of homologous genes in which a donor gene can replace a recipient gene. This exchange of DNA segments is called *recombination* and usually involves DNA from two different sources, but as we shall see later this is not always the case. Most recombination occurs between two DNA segments that are homologous; that is, they have similar nucleotide sequences. The sequence of events that

Fig. 5-22. Currently held view on how recombination takes place between homologous DNA strands.



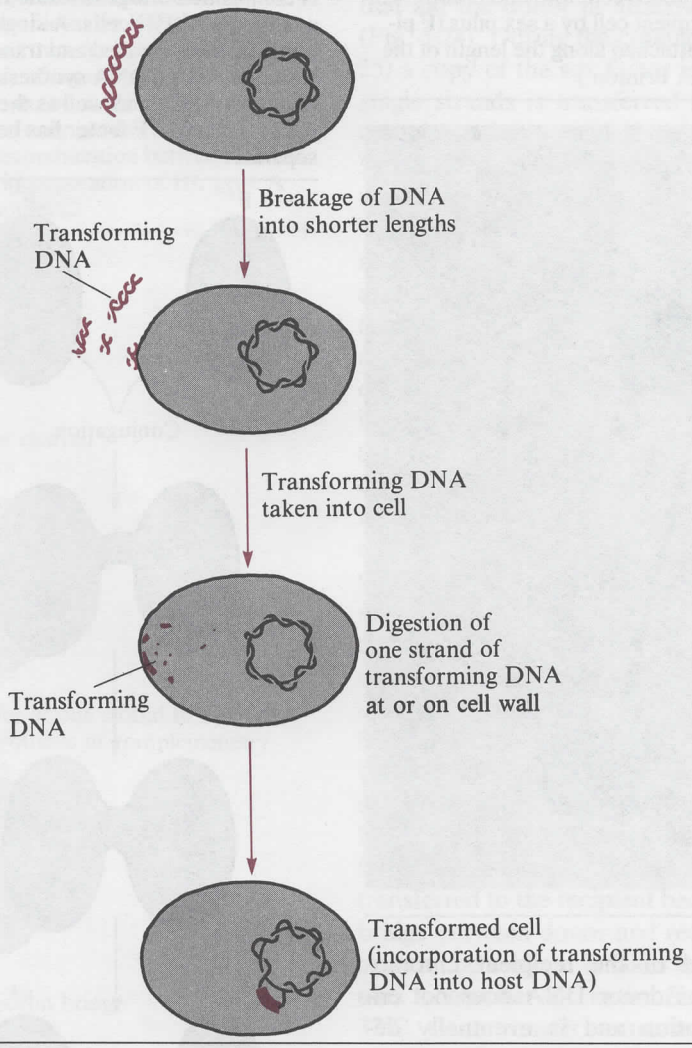
leads to the formation of recombinant DNA is diagrammed in Figure 5-22. The central feature of this model is that recombinant DNA molecules are formed by breaking and rejoining of intact DNA molecules of similar genotype and that recombination is independent of semiconservative DNA replication. Let us now examine the three mechanisms in which genes can be transferred.

Transformation

Transformation is the most primitive of the mechanisms for gene transfer among bacteria. The probability that transformation has contributed much to genetic variety is very small for most microorganisms. Transformation involves the taking up of cell-free fragmented DNA by a recipient cell and the recombination of genetic elements. Experimentally DNA is extracted from the donor cell and fragments of DNA are introduced with a population of viable recipient cells of a related species. The recipient cell will take up pieces of the DNA. Transformation has been studied primarily in gram-positive organisms such as *Bacillus subtilis* and species of *Streptococcus*. In gram-negative cells such as *E. coli* the cell is impermeable to DNA unless it is first treated with calcium chloride. The overall process of transformation may be divided into the following steps (Fig. 5-23). (These apply only to gram-positive bacteria. There are some variations when gram-negative bacteria are considered.)

1. Development of competence. *Competence* refers to the ability of a cell to take up DNA. Transformable bacteria become *competent* only under certain growth conditions. Log phase cultures (geometric growth) of streptococci, for example, release a protein *competence factor* that induces competence for the entire cell population.
2. Binding of DNA. Competent cells are believed to carry receptor sites for binding DNA. Only double-stranded DNA is bound; single-stranded

Fig. 5-23. Transformation process. See text for details of this process.



DNA or RNA is not bound. Homologous as well as heterologous (from a different species) DNA can be bound and can be taken up by the cell.

3. Uptake of DNA. DNA is taken up by the cell and converted into a DNase-resistant form. Double-stranded DNA is broken into shorter lengths, averaging 9×10^6 daltons, before uptake. The bound double-stranded DNA fragments are converted to single-stranded DNA. Single-stranded DNA, which has a molecular weight of between 3 and 5×10^6 daltons, is then taken up and bound to a protein in the cytoplasm that presumably protects it from nuclease activity.
4. Integration of DNA. Single-stranded donor DNA with sequences homologous to recipient DNA can enter into a recombination event. The donor single strand physically displaces a

Fig. 5-24. Conjugation in *Escherichia coli*. The larger, highly piliated cell is the donor cell (*top*) and is connected to the smaller recipient cell by a sex pilus (F pilus). Virus particles are attached along the length of the sex pilus. (Courtesy C. C. Brinton.)

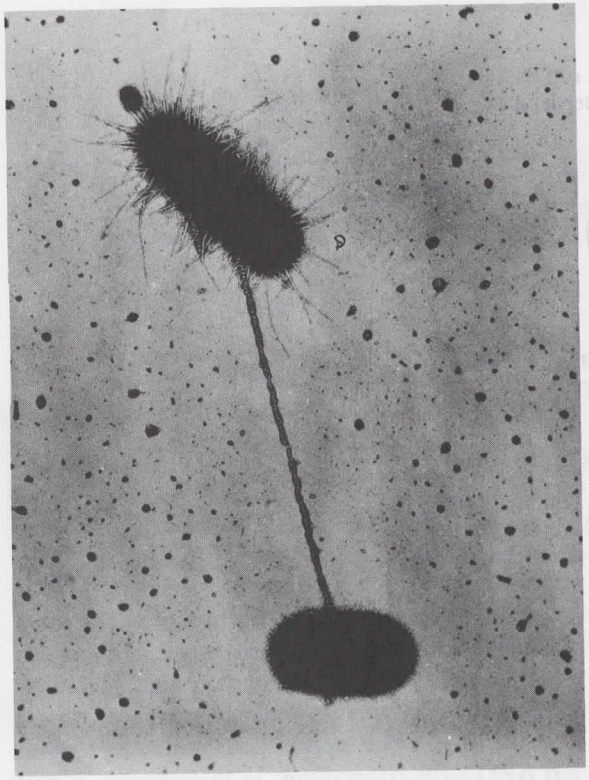
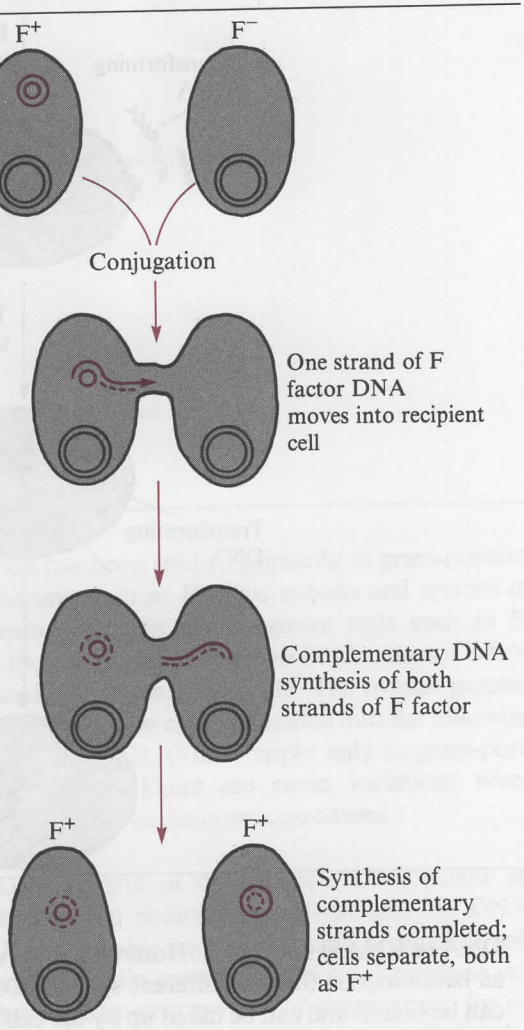


Fig. 5-25. Conjugation process involving $F^+ \times F^-$ cells. A conjugation bridge is formed between the donor (F^+) and recipient (F^-) cells. A single DNA strand of the donor F factor is cleaved and transferred to recipient. Complementary DNA synthesis occurs on the strand that is transferred as well as the strand remaining in the donor. Once the F factor has been transferred the cells separate.

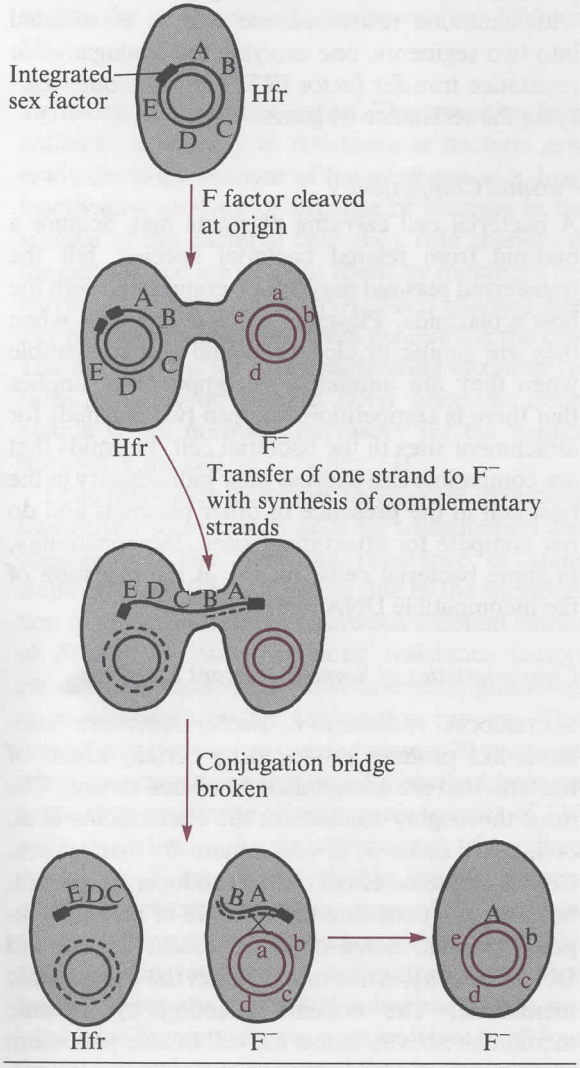


homologous strand of the recipient chromosome. Heterologous donor DNA does not engage in recombination and is eventually degraded by nucleases.

Conjugation

Conjugation is an important means of gene transfer, especially among the gram-negative bacteria. Only a few gram-positive cells are known to be involved in the conjugation process. Conjugation involves the interaction of two cell types referred to as the *donor* and the *recipient*. The genetic transfer between donor and recipient is mediated by an extrachromosomal piece of DNA called the *sex factor* or *fertility factor* (*F factor*) found in the donor cell. The donor cell is called F^+; the recipient cell, which does not contain the sex factor, is called F^-. The sex factor is double-stranded DNA that is about one-fortieth the size of chromosomal DNA.

Fig. 5-26. Conjugation process involving Hfr and F^- cells. Conjugation bridge is formed between donor (*Hfr*) and recipient (F^-) cells. Sex factor on Hfr chromosome is cleaved, and single-strand transfer is initiated. There is complementary synthesis of transferred DNA strand as well as remaining single strand in Hfr cell. The conjugation bridge is broken, and only genes A and B are transferred to recipient. Recombination between homologous genes results in the incorporation of Hfr gene A into the recipient chromosome.



It replicates autonomously. The F factor possesses several genes that code for the formation of sex pili that aid the donor in attaching to a recipient cell (Fig. 5-24). During the conjugation process (Fig. 5-25) a copy of the sex factor is made and one of its single strands is transferred to the recipient. The complementary strand of the donor single strand is synthesized in the recipient, which is now converted to an F^+.

HFR CELL FORMATION. The fertility factor does not always exist extrachromosomally but occasionally is integrated within the bacterial chromosome. In an integrated state the sex factor is under control of chromosomal replication. The cells possessing the integrated form of the sex factor are called *Hfr* (high frequency of recombination) cells. Hfr cells during conjugation use pili for attachment to recipient cells, but the sex factor is seldom transferred and most recipient cells remain F^-. In the donor, the F^+ factor can integrate at a number of different sites on the chromosome in different strains and can mobilize the transfer of chromosomal genes in different orders; for example, *a b c d e*, *b c d e*, or *d e f g* (Fig. 5-26). The reason for this is that the F^+ factor integrates at the origin (site of homology between chromosome and sex factor), and during conjugation some genes of the F factor are transferred first while the remaining genes are transferred last. Not all of the genes of the donor can be transferred to the recipient because the conjugation bridge between donor and recipient is very fragile and can be broken. It takes approximately 90 minutes to transfer the entire *E. coli* chromosome at 37°C. Gene transfer occurs in a specific order in a specific Hfr; therefore the order of genes on the bacterial chromosome can be determined. The remaining fertility factor genes are the last to be transferred, so recipient cells are seldom converted to Hfr or F^+ (Fig. 5-26).

Transduction

Transduction is a genetic transfer mechanism in which bacterial DNA is transported to recipient cells by a virus. This technique is discussed in Chapter 33.

CHARACTERISTICS OF EXTRACHROMOSOMAL DNA (PLASMIDS)

Extrachromosomal DNA includes accessory units of DNA that are not part of the host chromosome. Under most circumstances they are not essential for the successful growth and metabolism of their host. Some extrachromosomal DNA, however, does carry genes that supplement host activities and enable the bacterium to compete successfully with other organisms.

CHARACTERISTICS OF PLASMIDS

Plasmids are circular double-stranded molecules of DNA that have molecular weights varying from 1×10^5 to 1×10^8 daltons. The *E. coli* chromosome, for comparison, has a molecular weight of 2 to 3×10^9 daltons. Plasmids are capable of autonomous replication and will replicate at the same rate as chromosomal DNA, but there are circumstances in which they over-replicate, for example, during the conjugation process (see Conjugation earlier in this chapter). Plasmids can be divided into two classes, *conjugative* and *nonconjugative*, based on their ability or inability to initiate a conjugal act that permits the transfer of a plasmid copy from one cell to another. The characteristics of these two types are as follows:

1. A nonconjugative plasmid is able to replicate within the cell and can produce a number of copies of itself but lacks certain genes that promote transfer of itself to another bacterial cell, that is, the plasmid does not code for the formation of a sex pilus. Nonconjugative plasmids are found in gram-positive and gram-negative bacteria, and they can be transferred to other cells by transduction and transformation, the latter requiring special procedures. Nonconjugative plasmids in gram-negative cells can be transferred by conjugation only if another plasmid is present that contains the information for sex pilus formation, which is lacking in the nonconjugative plasmid.

2. A conjugative plasmid contains genes for pilus formation and permits transmission of copies of itself. The sex factor or fertility factor (F factor) and its relationship to conjugation are discussed under Conjugation. The plasmid carrying the sex factor genes may also carry genes containing information for antibiotic resistance and other factors. Conjugative plasmids are found almost exclusively in gram-negative bacteria. Some large conjugative plasmids, called *R factors*, carry genes associated with antibiotic resistance and can be dissociated into two segments, one carrying the conjugative or resistance transfer factor (RTF) and the other carrying the resistance (r) genes.

Plasmid Compatibility

A bacterial cell carrying plasmids may acquire a plasmid from related bacterial species, but the transferred plasmid may not be compatible with the host's plasmids. Plasmids are incompatible when they are similar or closely related and compatible when they are unrelated. Incompatibility implies that there is competition between two plasmids for attachment sites in the bacterial cell. Plasmids that are compatible can express their individuality in the host cell in the presence of other plasmids and do not compete for attachment sites. Incompatibility, in some bacterial cells, results in the digestion of the incompatible DNA molecule.

Characteristics of Some Important Plasmids

BACTERIOCIN PRODUCTION. Bacteriocins are antibiotic-like proteins produced by certain strains of bacteria that are antagonistic to related strains. The most thoroughly studied are the bacteriocins of *E. coli*, called *colicins*, of which there are many types. Colicins have no effect on the producer bacterium, but they can penetrate the cell wall of related competing strains. Some colicins possess RNase and DNase activity, while others affect the cytoplasmic membrane. The colicins affecting cytoplasmic membrane activity cause the cell to lose potassium and magnesium ions; this is believed to be the primary cause of death. The cells producing colicin are protected from its activity by the formation of

an immunity protein. The immunity protein forms a complex with the colicin and renders it inactive. How the colicin–immunity protein complex is inactivated is not known.

Bacteriocins are also produced by other bacteria—*pesticin* by *Yersinia pestis, staphylococcin* by *Staphylococcus epidermidis, butyricin* by *Clostridium butyricum*, and *pyocin* by *Pseudomonas aeruginosa*. Some of these bacteriocins show a great deal of specificity and have been used to type strains for epidemiologic purposes (see Chapter 13).

ANTIBIOTIC RESISTANCE FACTORS. The transition from antibiotic sensitivity to resistance in bacteria generally develops because of the synthesis of a drug-inactivating enzyme or because of changes in the surface of the bacterial cell. As a rule changes in the cell surface are brought about by mutations occurring in chromosomal DNA. Such changes usually prevent the antibiotic from penetrating the cell. The information for drug-inactivating enzymes resides primarily on plasmid DNA since this information is not normally necessary for the natural survival of the cell (see Chapter 11).

Drug resistance factors (R factors) are frequently transferred to microbial cells by transduction and conjugation. The penicillin resistance of some staphylococci, for example, is due to the transduction of penicillinase genes between different strains of *Staphylococcus*. Antibiotic resistance factors can also be transferred between enteric gram-negative organisms by the conjugation process. This type of transfer was postulated almost 20 years ago when Japanese scientists noted a marked increase in the drug resistance of pathogenic species of *Shigella dysenteriae*, the causative agent of bacillary dysentery. Some strains of this species demonstrated a resistance to one or more of the common chemotherapeutic agents such as chloramphenicol, streptomycin, tetracycline, and sulfonamide. What was alarming was that many individual strains isolated from human subjects were resistant to all four drugs. It was later discovered that in the intestinal tract of these people nonpathogenic *E. coli* also harbored multiple resistance factors. Experimentation revealed that the multiple resistance factors could be transferred in the conjugation process between different enteric microorganisms such as *E. coli* and *S. dysenteriae*. Prolonged antibiotic chemotherapy sustains multiply resistant microorganisms in the infected person, who may transmit them to someone who does not harbor antibiotic resistant species of bacteria.

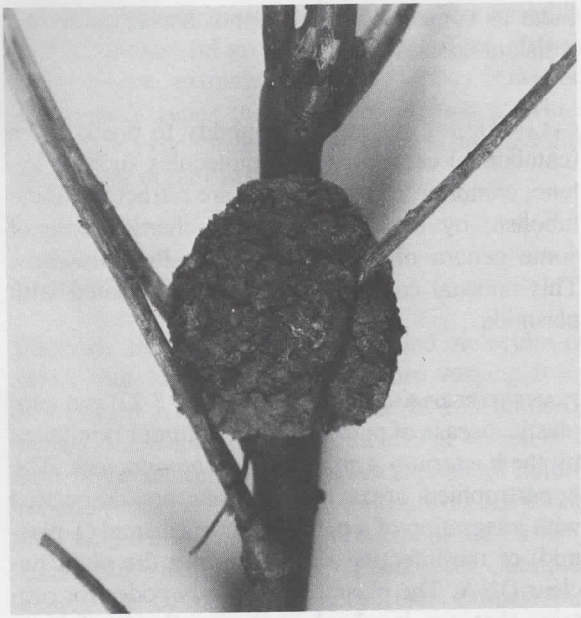
CATABOLIZING ACTIVITY. The ability to break down (catabolize) certain organic molecules such as xylene, camphor, or toluene that are refractory to catabolism by most organisms is characteristic of some genera of bacteria such as *Pseudomonas*. This unusual catabolic activity is associated with plasmids.

PLANT HYPERPLASIA. Crown gall (Fig. 5-27) is a neoplastic disease of plants in which a tumor is induced by the bacterium *Agrobacterium tumefaciens*. The hypertrophied areas of the plant are associated with integration of a part of a large plasmid (T plasmid) of the infecting bacterium into the plant nuclear DNA. The plasmid apparently codes for proteins that are involved in the synthesis of plant hormones that stimulate continued plant cell synthesis.

RESISTANCE TO METAL IONS. Heavy metal ions such as cobalt, mercury, and others are inhibitory to the growth of bacteria. There are plasmids, found primarily in the gram-positive staphylococci, that code for resistance to heavy metals. Metal ion plasmid resistance is often associated with antibiotic resistance factors, particularly penicillin resistance factors. Thus the transfer of metal ion resistance via plasmids from one cell to another is accompanied by penicillin resistance as well.

VIRULENCE DETERMINANTS. The ability of microorganisms to invade a host and cause disease is associated with special determinants carried on plasmids. These determinants often code for toxins that are detrimental to the host, but not always.

Fig. 5-27. Crown gall, a cancerous disease of plants caused by insertion of a portion of a plasmid carried by the bacterium *Agrobacterium tumefaciens*. (Courtesy of R. C. Lambe, Virginia Polytechnic and State University.)



The principal virulence determinants coded by plasmids are found in the following bacteria:

1. *E. coli*. Some *E. coli* strains produce two types of enterotoxin, designated heat labile (LT) and heat stable (ST), that are associated with disease and are plasmid-associated. In addition, these same strains produce pili that enable the cell to bind to intestinal epithelium. The pili are also plasmid-associated (see Chapter 23 for further details).
2. *Staphylococcus aureus*. An affliction of the skin of newborns called *scalded skin syndrome* is caused by certain strains of *S. aureus*. The disease is due to a toxin that is encoded on a plasmid (see Chapter 19 for further details).
3. *Clostridium perfringens*. One type of diarrheal disease in domestic animals is caused by *C. perfringens* type C. The disease is associated with a toxin that is encoded on a plasmid.
4. *Streptococcus* species. Several species of *Streptococcus* produce hemolysins (which lyse red blood cells) that are encoded on plasmid DNA (see Chapter 19 for further details).

OTHER ACCESSORY DNA ELEMENTS

Plasmids and viral DNA are accessory DNA elements that are capable of autonomous replication and confer important properties on the cell. There are other accessory DNA elements that are not capable of autonomous replication but nevertheless play an important role in genetic variability and in some instances the virulence of the organism. These accessory elements are called *insertion sequences* and *transposons*.

Insertion Sequences

Insertion sequences (IS) were discussed briefly earlier in this chapter under Spontaneous Mutations. Twenty or more IS elements have been found in the chromosome and plasmids of *E. coli*. They appear to contain only information for inserting themselves into various regions of the DNA. IS elements possess inverted repeat sequences, referred to as *palindromic sequences*, at their ends (Fig. 5-28). These inverted repeats are apparently recognized at their points of insertion. For example, the F factor and the bacterial chromosome contain common nucleotide sequences that are insertion sequences. The location of the specific chromosomal IS element thus determines the location of the site for insertion of the sex factor and conversion of the bacterial cell to Hfr. The transfer of an IS element to another location on the DNA molecule is called *transposition*. The effect of IS elements on gene activity was discussed previously.

Transposons

The movement of IS elements also affects the movement of another accessory DNA element called a *transposon*. Transposons consist of IS elements that are joined to other genes (Fig. 5-29). Thus the movement of the transposon involves

Fig. 5-28. Characteristics of the terminal sequences of insertion sequence elements. The inverted repeat sequences are called palindromic sequences.

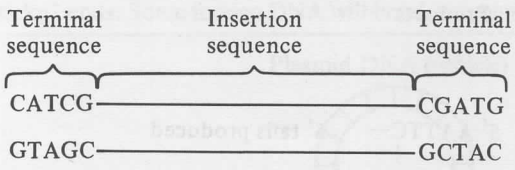
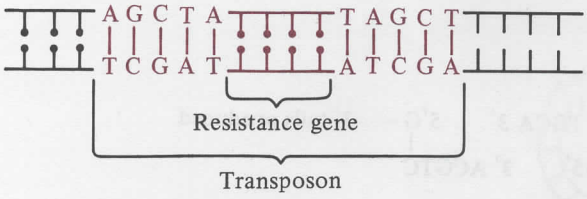


Fig. 5-29. Hypothetical structure of a transposon. Transposon consists of inverted repeat sequences that enclose genetic information such as antibiotic resistance genes.



movement of IS elements as well as genes. The genes found to be associated with the transposon are antibiotic resistance genes. Transposons can move from one plasmid to another and from plasmid DNA to chromosomal DNA and vice versa. The multiple antibiotic resistance genes found in some plasmids are believed to have evolved by movement of transposons. Transposons can insert into other DNA molecules with great frequency and do not require the same factors that are involved in homologous DNA-DNA interactions.

RESTRICTION ENDONUCLEASES AND RECOMBINANT DNA

We have previously discussed the genetic mechanism of recombination; however, the term *recombinant DNA* has a slightly different meaning. *Recombinant DNA* refers to the creation of DNA molecules by the association of DNA molecules derived from different biological sources. Recombinant DNA technology is commonly called *gene splicing* or *genetic engineering*. The basis of recombinant DNA technology is restriction endonucleases found in bacteria. The term *restriction* refers to the fact that the enzymes are capable of degrading certain foreign DNA molecules. The host's DNA is protected from endonuclease attack by modification enzymes that methylate host DNA and thus make it resistant to restriction enzymes. Over 80 restriction enzymes that recognize specific sequences in the DNA molecule have been discovered. The types used for gene splicing recognize a specific sequence and cut the DNA within the recognition sequence. This type of recognition is illustrated in Figure 5-30. As you can see from this figure, complementary tails that are said to be "sticky" are produced on the DNA strands. In other words, they will have a tendency to reattach (anneal). Regardless of the DNA molecule a single restriction enzyme is specific, and the same sticky ends are always produced. Thus, if you had DNA from bacteria, yeast, or plant and each was treated with the same restriction enzyme, the palindromic recognition sites would be the same. If treated DNA from different sources is placed together, recombinant DNA molecules can be produced. A sequence of steps is used in recombinant DNA technology. For our example we will assume that we wish to produce a recombinant DNA molecule that contains eukaryotic and prokaryotic DNA (Fig. 5-31).

1. Eukaryotic DNA is treated with a specific restriction endonuclease, and the sticky segments are isolated. These segments are called *passenger DNA*.
2. Plasmid DNA will serve as the *vehicle* or vector for carrying the eukaryotic DNA segments. The vehicle DNA is usually a bacterial plasmid, but a phage could also be used. The vector DNA is treated with the same restriction endonuclease as the passenger DNA. We have now produced a vector with sticky tails that can hybridize with the sticky tails of eukaryotic DNA segments.

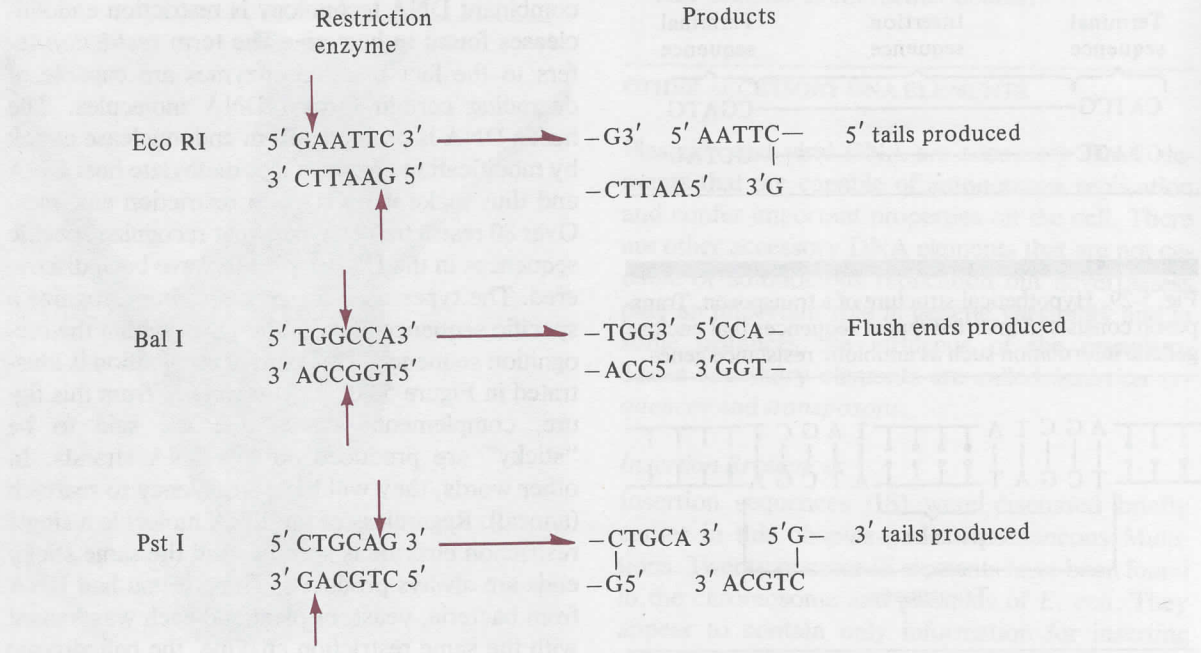


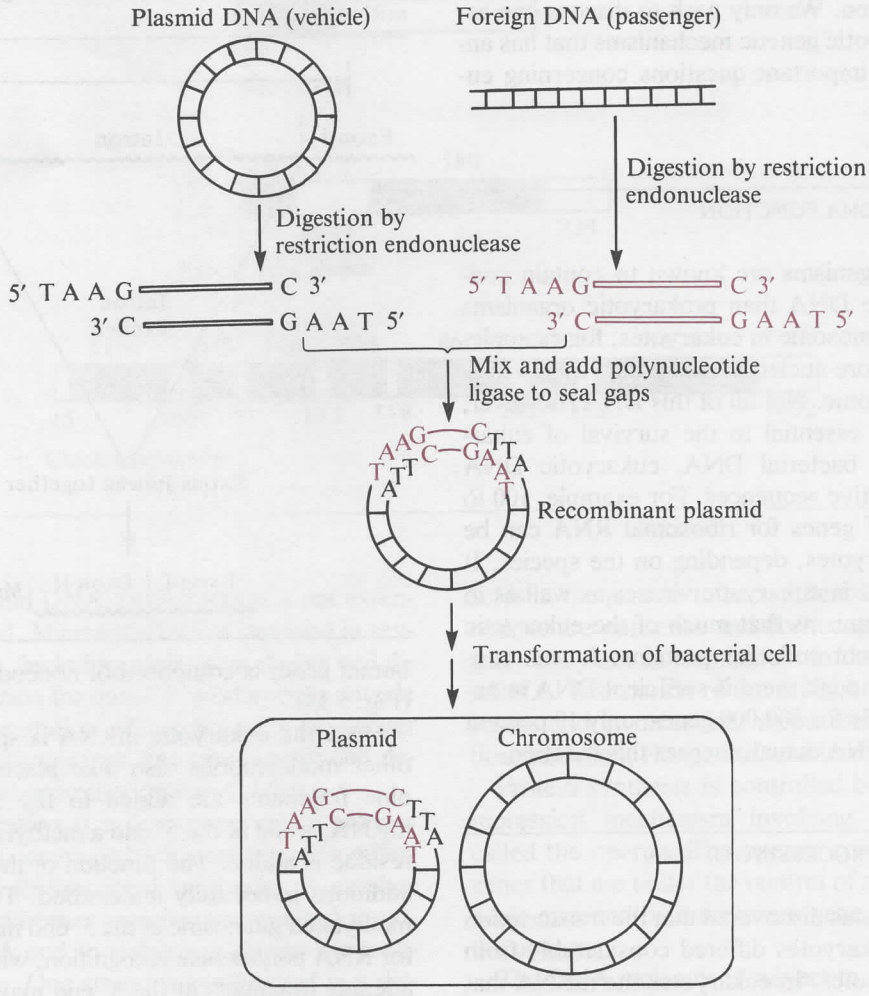
Fig. 5-30. Recognition sites for some restriction endonucleases. Arrows point to sites of cleavage. The endonucleases were obtained from microorganisms: Eco RI, *Escherichia coli*; Bal I, *Brevibacterium albidum*; and Pst I, *Providencia stuarti*.

3. Once the eukaryotic DNA segments are inserted into the vector a DNA ligase seals the open ends and a circular hybrid plasmid is produced.
4. Once the hybrid plasmid is produced we are now ready to insert it into a host cell in which the recombinant DNA molecule will replicate with host DNA. This process is referred to as *cloning*. The insertion process is actually a transformation of *E. coli*. *E. coli* is treated with $CaCl_2$, which permits uptake of the hybrid plasmid.
5. Transformed *E. coli* cells will contain recombinant plasmids containing various eukaryotic genes. The trick is to identify the transformed *E. coli* that contains the eukaryotic gene of interest. One useful technique is to use a vector that codes for an easily assayed property such as resistance to an antibiotic that is inactivated by insertion of a DNA fragment into the vector.

Importance of Recombinant DNA Technology

Recombinant DNA technology has tremendous application to basic science as well as applied areas of science. Gene cloning has provided geneticists with a tool for the mutation and manipulation of organisms far more powerful than classic methods of genetic manipulation, which permit the analysis of only certain aspects of the function of any gene. For example, it is now possible to isolate mutants in which the mutation is lethal, a technique that was impossible with classical mutation techniques. The applications of recombinant DNA technology are practically limitless, and many previously speculated possibilities are now coming to fruition. Gene manipulation is being exploited to create products such as hormones, vaccines, enzymes, and pharmaceuticals that can be produced by bac-

Fig. 5-31. Recombinant DNA experiment. Plasmid DNA (bacterial source) plus foreign DNA (eukaryotic source, for example) are digested by endonuclease. DNA fragments are produced with complementary "sticky" ends. Some foreign DNA will bond with plasmid DNA. The plasmid circularizes and can be used to transform a bacterial cell. The foreign DNA (gene) can be expressed in the bacterium. (From R. F. Boyd, *General Microbiology*. St. Louis: Mosby, 1984.



terial fermentations. Animal products such as insulin and mammalian growth hormone are now produced in bacterial cells and are commercially available. Many industrial wastes constitute a major environmental pollution problem. Experiments are now underway to create bacteria with special degradative properties. Some wastes can, in principle, be converted to useful products such as single-cell protein that can be used as animal feed or can be converted to methane, a source of energy. Food yields could also be improved by manipulation of nitrogen-fixing bacteria so they can be utilized by plants other than the leguminous ones.

EUKARYOTIC GENETICS

It is not our purpose to discuss all the aspects of eukaryotic genetics. They are more appropriately discussed in other courses, which most of you have previously taken. We only wish to discuss one aspect of eukaryotic genetic mechanisms that has answered some important questions concerning eukaryotic DNA.

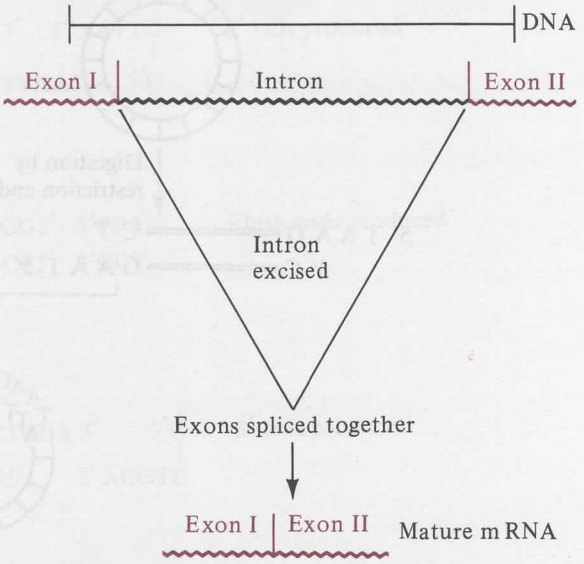
EUKARYOTIC DNA FUNCTION

Eukaryotic organisms are known to contain considerably more DNA than prokaryotic organisms do. Each chromosome in eukaryotes, for example, contains 10^6 more nucleotide pairs than does a bacterial chromosome. Not all of this DNA, however, appears to be essential to the survival of eukaryotes. Unlike bacterial DNA, eukaryotic DNA contains repetitive sequences. For example, 100 to 1000 copies of genes for ribosomal RNA can be found in eukaryotes, depending on the species. It was discovered in eukaryotic viruses as well as in eukaryotic organisms that much of the eukaryotic genome does not code for proteins. It was concluded that although there is sufficient DNA in eukaryotes to code for 500,000 genes, only 10 percent or less of the DNA actually serves this function.

EUKARYOTIC PROCESSING OF RNA

In the 1970s it was discovered that the transcription of DNA in eukaryotes differed considerably from that in prokaryotes. In eukaryotes the mRNA that is transcribed from DNA is considerably longer than the mature mRNA that is eventually translated. It was realized that two types of sequences are found in the mRNA: those that code for information (*exons*) and those that do not (*introns*). During the formation of mature mRNA the introns, or noncoding nucleotide sequences, are excised, and the exons are spliced together (Fig. 5-32). As much as 70 percent of some genes, such as the chick ovalbumin gene, is composed of noncoding sequences (Fig. 5-33).

Fig. 5-32. Mechanism of mRNA processing in eukaryotic systems. A genetic sequence on the DNA is transcribed into an RNA molecule some of which contains genetic information (exons) and some which does not (introns). The intron (or introns) is (are) excised and the exons spliced together to produce a single mature mRNA transcript.



Once the eukaryotic mRNA is spliced together other modifications also take place. Poly-A adenine fragments are added to the 3′ end of the mRNA, while at the 5′ end a methylated guanosine residue is added. The function of these nucleotide additions is not fully understood. The addition of methylated guanosine at the 5′ end may act as a site for RNA polymerase recognition, while the poly-A adenine fragments at the 3′ end may be important in mRNA stabilization.

SUMMARY

DNA in the microbial cell can be divided into chromosomal DNA, or that required for survival of the

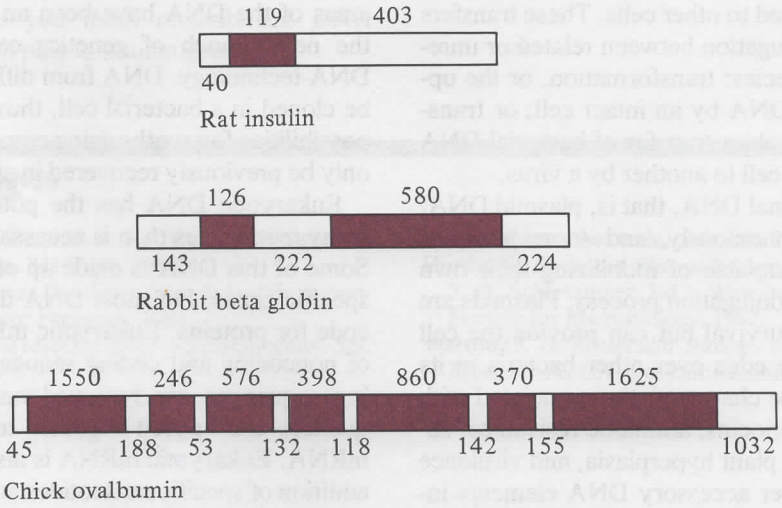


Fig. 5-33. Intervening sequences in various eukaryotic genes. The numbers indicate nucleotides present in various introns and exons. (□ = intron; □ = exon.) (Adapted from W. S. Klug and M. R. Cummings, *Concepts of Genetics*. Columbus, OH: Merrill, 1983.)

cell, and plasmid DNA, or that which is not essential for survival. Microbial DNA is involved in several processes, including replication during cell division, replication for transfer to other cells outside cell division, mutation, translation of a messenger RNA (mRNA) transcript into proteins for cell development, and control of protein synthesis.

DNA replication is a semiconservative process that in the cell occurs on both strands and in different directions (bidirectional synthesis). A number of enzymes and other proteins are required to unwind the DNA and to stabilize it during the replication process. One strand is synthesized in a discontinuous manner; the other, in a continuous manner.

DNA is transcribed into a mRNA molecule, which is translated into proteins. Messenger RNA represents a transcription of genes on the DNA. The information coded in the gene is in the form of triplets of bases, with each triplet (codon) specifying an amino acid. It is the sequence of triplets in the gene that determines the sequence of amino acids in the protein to be synthesized. Some of the codons are nonsense and do not specify an amino acid. They act as terminators in the reading of the mRNA. Translation of the mRNA occurs on ribosomes and involves four steps: amino acid activation, initiation, elongation, and termination.

Protein synthesis is controlled by an induction-repression mechanism involving a genetic unit called the operon. The operon contains structural genes that are under the control of a repressor molecule encoded by a repressor gene located outside the operon.

The DNA molecule is subject to alterations (mutations) that may or may not affect the phenotype of the cell. Mutations are of two types, spontaneous and induced. The agents causing induced mutations are called mutagens and include both chemical and physical agents. Spontaneous mutations may be due to changes in the electrochemical nature of the base or to insertion sequence elements. Phenotypic changes can be brought about by mutations that are missense, nonsense, or

frameshift. The most commonly used technique to isolate mutants is called replica plating. The Ames test is a technique for determining the potential mutagenic effect of various chemicals.

DNA can engage in homologous recombination when it is transferred to other cells. These transfers can be due to conjugation between related or unrelated microbial species; transformation, or the uptake of pieces of DNA by an intact cell; or transduction, which involves transfer of bacterial DNA from one bacterial cell to another by a virus.

Extrachromosomal DNA, that is, plasmid DNA, can replicate autonomously, and some plasmids (conjugative) are capable of mobilizing their own transfer as in the conjugation process. Plasmids are not essential for survival but can provide the cell with a competitive edge over other bacteria in its environment. Some characteristics associated with plasmids are bacteriocins, antibiotic resistance, catabolizing activity, plant hyperplasia, and virulence determinants. Other accessory DNA elements include insertion sequences (IS) and transposons. IS elements code for no genes, while the larger transposons often carry antibiotic resistance genes. IS elements and transposons can move from one DNA molecule to another.

Restriction endonucleases that can cut specific areas of the DNA have been an important tool in the new branch of genetics called recombinant DNA technology. DNA from different sources can be cloned in a bacterial cell, thus opening up wide possibilities for synthesizing compounds that could only be previously recovered in small quantities.

Eukaryotic DNA has the potential to code for many more genes than is necessary for its survival. Some of this DNA is made up of several copies of specific genes, but most DNA does not appear to code for proteins. Eukaryotic mRNA is composed of noncoding and coding sequences. The noncoding sequences are removed and the coding sequences are spliced together to make a mature mRNA. Eukaryotic mRNA is also modified by the addition of specific nucleotides at the 3′ and 5′ ends of the molecule.

QUESTIONS FOR STUDY

1. In what ways does plasmid DNA differ from chromosomal DNA? In what ways are they the same?
2. What proteins are required for unwinding of the DNA? What enzymes are required for replication?
3. Distinguish between nonsense, missense, and frameshift mutations.
4. What is meant by the degeneracy of the genetic code?
5. What are the P and A sites on the ribosome? How are they involved in protein synthesis?
6. What genes are part of the operon? What is the relationship of the repressor gene to the genes of the operon?
7. How are insertion sequences capable of causing mutations?
8. How do tautomers give rise to spontaneous mutations? How is the genotype changed?
9. What is the mechanism by which ultraviolet light causes mutations?
10. What is the distinction between an auxotroph and a prototroph?
11. Describe the Ames test for determining the potential carcinogenicity of chemicals.
12. What are the ways in which DNA can be transferred from cell to cell?
13. Describe the sequence of events associated with genetic transformation.
14. How does an Hfr cell differ from an F^+ cell in the conjugation process?
15. What are some of the important plasmids found in bacteria?

16. How does a transposon differ from an insertion sequence?
17. What is the difference between vehicle and passenger DNA in recombinant DNA technology?
18. What role do you think recombinant DNA technology will play in human genetics?
19. What are the differences between prokaryotes and eukaryotes in mRNA transcription?
20. What is the difference between an exon and an intron?

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6. ENERGY METABOLISM

OBJECTIVES

To briefly explain the sources of energy available to microbial cells

To briefly describe the process of glycolysis, indicating the important intermediates and end products

To explain the difference between substrate level phosphorylation and electron transport phosphorylation as means of producing ATP

To briefly explain the hexose monophosphate shunt and its importance in metabolism

To briefly explain how ATP formation is coupled to electron transport

To describe the overall purpose of the Krebs' cycle in metabolism

To explain the distinction between fermentation and respiration

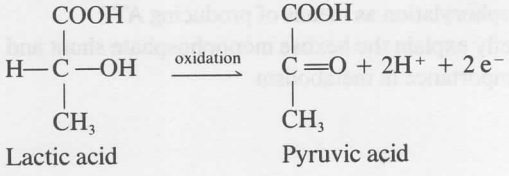
To define: dehydrogenation, catabolism, anabolism, chemotroph, phototroph, beta oxidation, deamination, oxidation-reduction potential

Oxidation-Reduction and Energy
Energy Sources
Light Energy
Chemical Energy
Organic Energy Sources
Inorganic Energy Sources
Energy Production in the Cell
Fermentation
Glycolysis
Other Pathways of Glucose Catabolism
Hexose Monophosphate Shunt
Entner-Doudoroff Pathway
Other Fermentation Processes
Respiration
Aerobic Respiration
Electron Transfer During Aerobic Respiration
Electron Transport Phosphorylation
Coupling of ATP Formation to Electron Transport
Major Sources of Electrons
Anaerobic Respiration

It is difficult to imagine that a bacterial cell, as small as it is, carries out many of the same biochemical reactions that occur in our own bodies. Glycolysis, amino acid biosynthesis, protein synthesis, and the like, with slight variations, are the same in bacteria as they are in humans and other animals. All of these organized chemical activities within the cell are collectively called *metabolism*. Some are involved in generating energy by breaking down inorganic and organic compounds (*catabolism*); others are associated with the use of energy or synthesis of organic compounds (*anabolism*). Reactions within the cell are in some way involved in either energy production or energy use. This chapter describes the energy sources available to bacterial cells and how energy can be extracted from them.

OXIDATION-REDUCTION AND ENERGY

Organisms that use chemical energy do so by engaging in oxidation-reduction reactions. The term *oxidation* implies that oxygen is involved in the reaction (Fig. 6-1), but that is not always true. Oxidation-reduction reactions involve the transfer of electrons and sometimes electrons plus protons (H^+). *Oxidation* is defined as the removal of electrons from a substance, while the term *reduction* means the gain of electrons by a substance. When electrons are removed from a substance in the cell they do not remain free in the cytoplasm but instead are gained by another substance. The substance that donates electrons is the energy source—the more electrons that can be donated the greater the potential energy of that source. Oxidation in biological systems, in which organic molecules are involved, usually means the loss of an electron and proton together, that is, a hydrogen atom (H). For this reason these oxidations are described as *dehydrogenations*, for example:



Lactic acid Pyruvic acid

In the above reaction, lactic acid is oxidized by the removal of two protons and two electrons to a more oxidized compound called pyruvic acid. The pyruvic acid is capable of further oxidation, but it does not have the energy potential of lactic acid. The two protons and electrons do not remain free in the cytoplasm but will be picked up, usually together, by an acceptor molecule. These proton and elec-

Fig. 6-1. Oxidation reaction in which oxygen is involved.

$$4\,Al + 3O_2 \longrightarrow 2\,Al_2O_3$$

Fig. 6-2. Oxidation-reduction potential of some important biological oxidation-reduction pairs. Oxidation-reduction potential is measured in electron volts. Arrow at right shows direction of normal flow of electrons (*e*) in which energy is released during oxidation. Negative values for electron volts indicate a greater electron pressure and the ability of the pair to donate electrons. Positive values for electron volts indicate less electron pressure and the tendency to accept electrons. (NAD = nicotinamide-adenine dinucleotide; NADH = reduced form of NAD; FAD = flavin-adenine dinucleotide; FADH = reduced form of FAD.)

Oxidation-reduction pairs	Electron volts (pH 7.0)
$H_2 \rightleftharpoons 2H^+ + 2e^-$	−0.414
$NADH + H^+ \rightleftharpoons NAD + 2H^+ + 2e^-$	−0.32
$FADH + H^+ \rightleftharpoons FAD + 2H^+ + 2e^-$	−0.22
Lactate $\rightleftharpoons$ pyruvate $+ 2H^+ + 2e^-$	−0.19
Succinate $\rightleftharpoons$ fumarate $+ 2H^+ + 2e^-$	+0.031
Cytochrome $a_3^{2+} \rightleftharpoons$ cytochrome $a_3^{3+} + e^-$	+0.285
$NO_2 + H_2O \rightleftharpoons NO_3 + 2H^+ + 2e^-$	+0.421
$2H_2O \rightleftharpoons O_2 + 4H^+ + 4e^-$	+0.816

tron acceptors will be discussed shortly.

The oxidation of lactic acid to pyruvic acid can be described as an *oxidation-reduction system* when an enzyme catalyzes the reaction. There are a number of these oxidation-reduction pairs in biological systems, and each is measured in electron volts (Fig. 6-2). As you can see from Figure 6-2 there are negative and positive oxidation-reduction potentials for the pairs illustrated. The natural flow of electrons is from a more negative substance to a more positive one. In other words all the systems with negative values can donate electrons to those systems that are more positive than they are. The greater the difference in oxidation-reduction potential between the two systems the greater the amount of energy that can be realized. For example, if oxygen is going to be our final electron acceptor more energy can be realized from the oxidation of H_2 than the oxidation of NO_2. The potential difference in the oxidation of H_2 is $+0.816 - (-0.414)$ or 1.230, while for NO_2 it is $+0.816 - (+0.421)$ or 0.395. To transfer electrons from a more positive oxidation-reduction pair to a more negative oxidation-reduction pair requires an input of energy. Those oxidation-reduction pairs that are somewhere in between the more negative and positive ones, for example, succinate-fumarate, can act as electron donors as well as electron acceptors. The succinate-fumarate pair can donate electrons to cytochrome a_3 and can accept electrons from the NADH + H^+–NAD pair (NAD = nicotinamide-adenine dinucleotide; NADH = reduced form of NAD).

ENERGY SOURCES

Energy is required to carry out the many biochemical and biophysical processes that occur in the cell. This energy may be supplied to the cell in basically two different forms. Photosynthetic organisms (*phototrophs*) take in energy in the form of radiations from the sun and convert it to chemical energy. Nonphotosynthetic organisms (*chemotrophs*) extract energy from chemical compounds (inorganic or organic molecules).

LIGHT ENERGY

Photosynthesis is a process common to plants and algae, but many bacteria (Cyanobacteria, purple and green bacteria) also use this process. The ability of these microorganisms to photosynthesize is related to the presence of pigment molecules resembling chlorophyll. The photosynthetic process consists of many reactions but is divided primarily

into two types of reactions: those that occur in the dark and those occurring in the light. In light the electromagnetic radiations of the sun are converted to chemical energy, while in the dark the chemical energy is used to produce organic molecules. Photosynthetic bacteria are not infectious, and we will not discuss them further.

CHEMICAL ENERGY

Organic Energy Sources

Organic energy sources include a multitude of compounds ranging from simple two-carbon molecules such as acetate to more complex ones such as lipids, which may contain 40 to 50 carbon atoms. The ability of the cell to use a variety of energy sources is related to its genetic potential and production of specific catabolic enzymes. An organism's potential to live in a certain habitat is dependent on these energy sources. Parasitic microorganisms are chemoheterotrophic (organic compounds are their energy and carbon sources), and their energy source is usually the same as their carbon source. Glucose ($C_6H_{12}O_6$), for example, is a common carbon and energy source for most bacteria.

Inorganic Energy Sources

Inorganic sources of energy are used primarily by microorganisms that inhabit the soil and water. These sources of energy include hydrogen gas (H_2), hydrogen sulfide (H_2S), nitrate (NO_3), iron (Fe^{2+}), and others. Their oxidation also includes electron transfer, but protons are not directly involved. The electron acceptor for microorganisms using inorganic energy sources in most instances is oxygen. For example, hydrogen gas is used by hydrogen bacteria to produce energy during the following oxidation reaction:

$$H_2 + \tfrac{1}{2}\,O_2 \longrightarrow H_2O$$

ENERGY PRODUCTION IN THE CELL

Some of the energy obtained from food must be conserved in a chemical form; otherwise it would be dissipated as heat and thus wasted to the cell. In the biological world chemical energy to be used for various cellular activities can be found in only a few organic phosphates such as adenosine triphosphate (ATP). The energy in the ATP molecule can be used by the cell for such processes as biosynthesis, movement, and transport of material across the cytoplasmic membrane.

The major problem confronting the cell is, How can energy be produced and conserved during the oxidation of foodstuffs? A considerable amount of energy is contained in the highly reduced carbohydrate glucose. This potential energy must be extracted by the cell in a series of controlled reactions rather than in a single oxidation burst. If released in a one-step process much of the energy would be largely dissipated as heat because only a small portion could be trapped in organic molecules such as ATP. Large bursts of energy as heat would lead to a number of harmful physiological responses.

Energy can be produced in the cell by two kinds of oxidation processes: *fermentation* and *respiration*. Enzymatic reactions in each process engage in the release and capture of energy. Electrons removed during fermentation are accepted by an organic molecule such as pyruvic acid, but in respiration the final electron acceptor is an inorganic molecule, often oxygen. Both processes generate energy and trap it in the form of ATP, but they differ somewhat in the way they accomplish the task.

FERMENTATION

Fermentation is an anaerobic process, and for those organisms that can survive only in the absence of air (obligate anaerobes) fermentation is a major source of energy. Those organisms that can grow in the presence or absence of air (facultative anaerobes) use fermentation as a source of energy only when oxygen is absent. Organisms that are to-

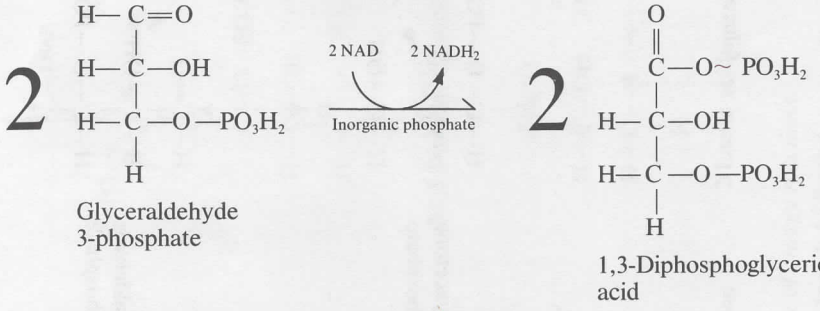
tally dependent on the presence of air (obligate aerobes) use aerobic respiration to obtain their energy.

Glycolysis

The special fermentation process in which glucose is anaerobically oxidized to two molecules of pyruvic acid is called *glycolysis*, or the *Embden-Meyerhof-Parnas (EMP) pathway*. The reactions in glycolysis can also be used by aerobic organisms, which link an anaerobically produced molecule to an aerobic set of reactions called the *tricarboxylic acid cycle* or *Krebs cycle*. These are discussed under Aerobic Respiration in this chapter. The reactions involved in glycolysis are illustrated in Figure 6-3. The student should not be overwhelmed by the chemical formulas but merely try to understand the overall function of glycolysis. A brief characterization of the reactions is as follows:

Reactions 1 to 4. Glucose, a six-carbon molecule, is phosphorylated, rearranged, and cleaved into two three-carbon units called *glyceraldehyde 3-phosphate*. In that series of reactions, two molecules of ATP are required; that is, energy is needed to push the reaction that far.

Reaction 5. Two molecules of glyceraldehyde 3-phosphate are phosphorylated to two molecules of 1,3-diphosphoglyceric acid.[1] This step represents the first oxidation reaction, and there is a transfer of electrons and hydrogens to NAD, the first acceptor molecule. In this oxidation of an aldehyde to an acid, the bond energy extracted is not lost but is captured by inorganic phosphate to form a high-energy phosphate bond represented as ~ on carbon 1 of the 1,3-diphosphoglyceric acid. This process of phosphate transfer and synthesis of ATP is called *substrate-level phosphorylation*. It involves the oxidation of an organic substrate, glyceraldehyde 3-phosphate, by an oxidant, NAD, to generate an intermediate with a high energy of hydrolysis. The transfer of energy occurs with the help of cytoplasmic enzymes. This is to be contrasted with electron transport phosphorylation, which occurs on membranes and will be discussed later.

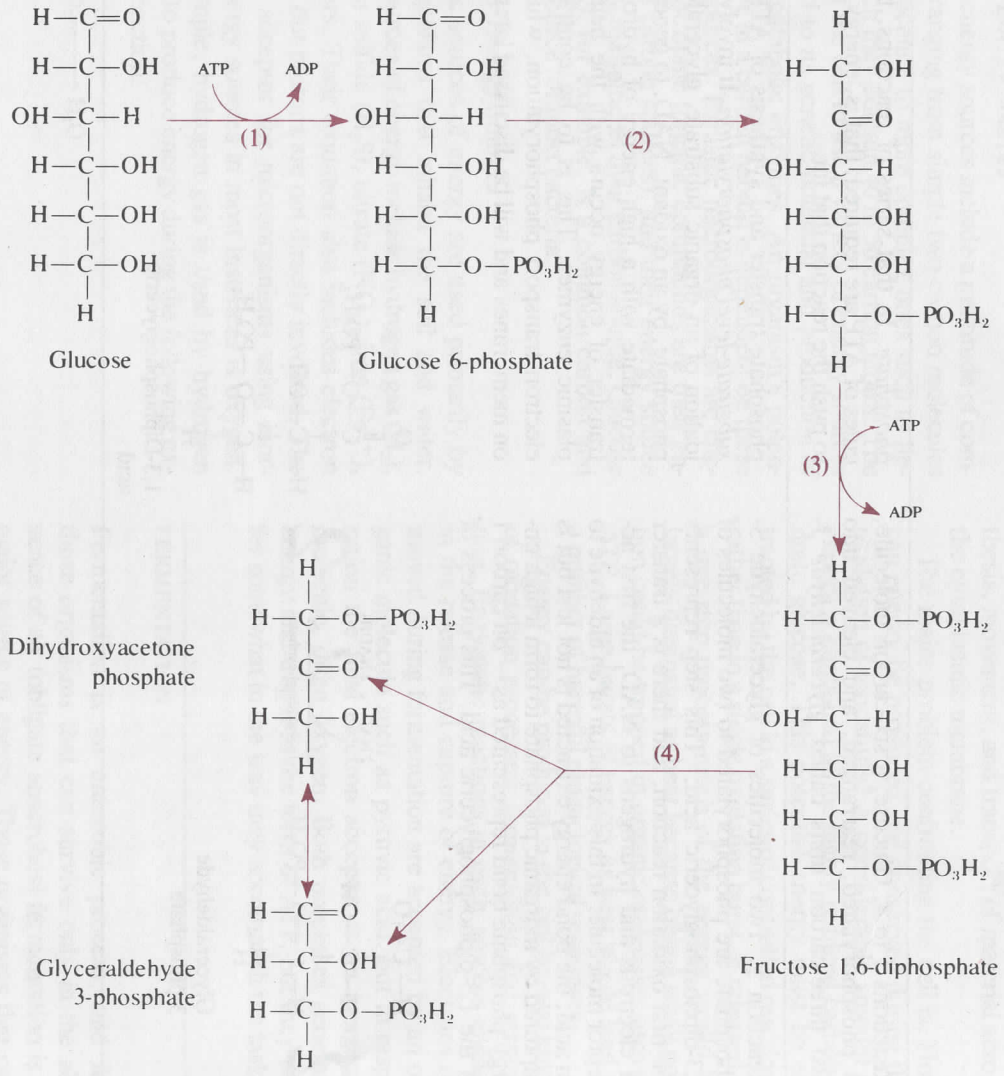


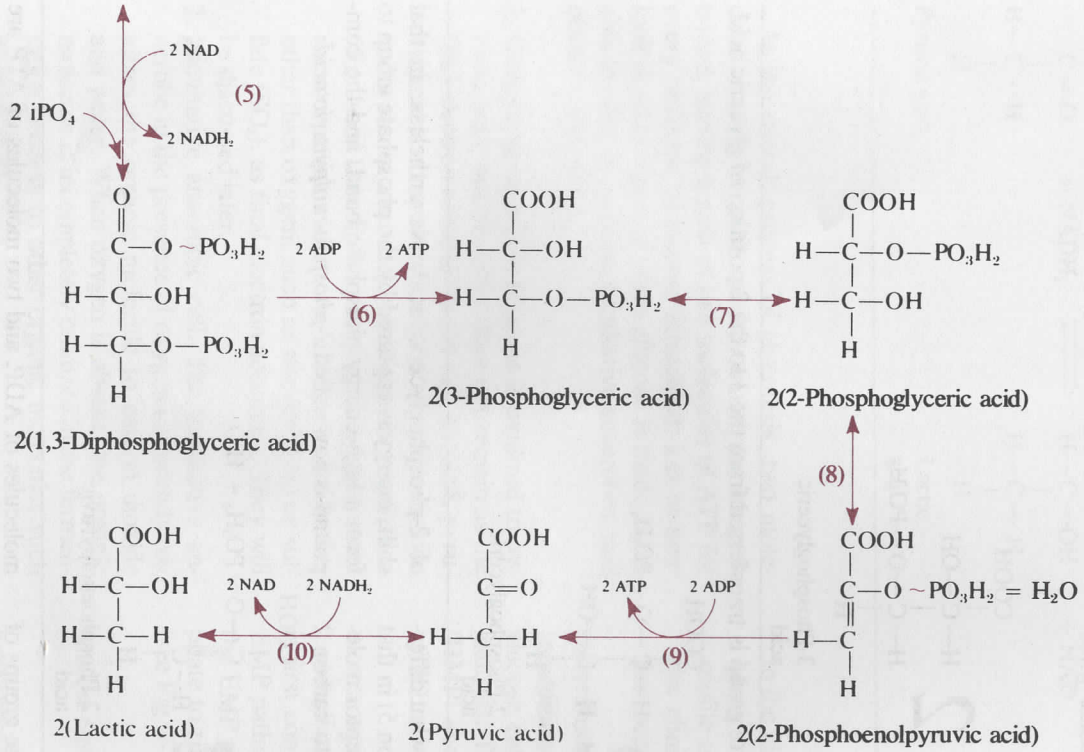
Glyceraldehyde 3-phosphate

1,3-Diphosphoglyceric acid

[1]This is not quite the case, as reaction 4 (Fig. 6-3) demonstrates. One molecule of dihydroxyacetone phosphate is produced in addition to one molecule of glyceraldehyde 3-phosphate. A separate enzyme catalyzes the conversion of the dihydroxyacetone phosphate to another molecule of glyceraldehyde 3-phosphate. In reactions 5 to 10, therefore, all reactants and products are represented as containing two molecules of each component.

Fig. 6-3. Sequence of reactions occurring in glycolysis or the Embden-Meyerhof-Parnas pathway. The numbers below or beside the reaction arrows correspond to the reaction numbers in the text.



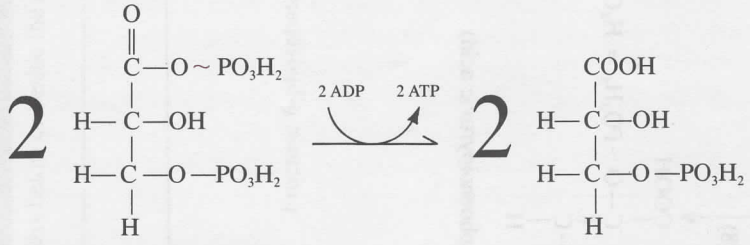


Summary of energy reactions

Reaction 1 — 1 ATP used
Reaction 3 — 1 ATP used
Reaction 6 — 2 ATP generated
Reaction 9 — 2 ATP generated

Sum: 4 ATP generated and 2 ATP used equals 2 ATP gained per molecule of glucose fermented.

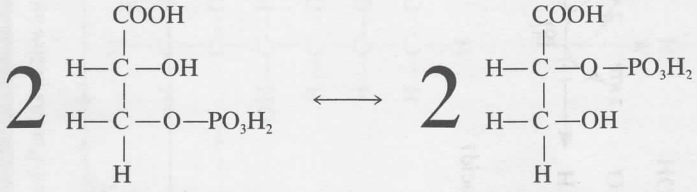
Reaction 6. The energy in the phosphate group of the two molecules of 1,3-diphosphoglyceric acid is transferred to two molecules of adenosine phosphate (ADP), and two molecules of ATP are generated.



1,3-Diphosphoglyceric acid

3-Phosphoglyceric acid

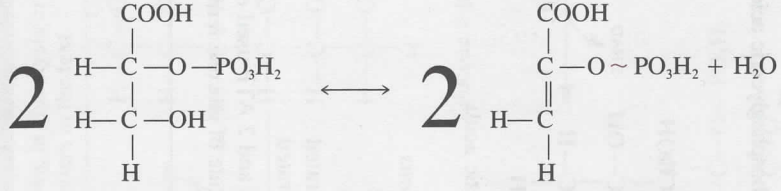
Reaction 7. In this reaction the phosphate group is transferred from the 3 to the 2 position of glyceric acid.



3-Phosphoglyceric acid

2-Phosphoglyceric acid

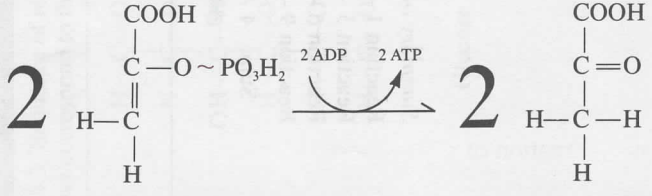
Reaction 8. This oxidation step is somewhat different from the first oxidation step (reaction 5) in that electrons are not transferred to the acceptor molecule but merely shifted from carbon 1 to carbon 2 of 2-phosphoglyceric acid. Nevertheless, in that shift, energy is captured by the phosphate group to form a high-energy phosphate bond, and the compound is now called *2-phosphoenolpyruvic acid*.



2-Phosphoglyceric acid

2-Phosphoenolpyruvic acid

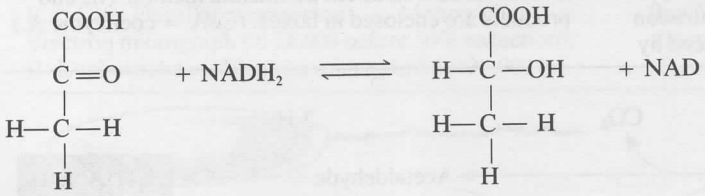
Reaction 9. The energy of the phosphate groups of 2-phosphoenolpyruvic acid is transferred to two molecules of ADP, and two molecules of ATP are generated.



2-Phosphoenolpyruvic acid

Pyruvic acid

Reaction 10. A regeneration of NAD is accomplished by the reduction of pyruvic acid.



Pyruvic acid — Lactic acid

In the overall process of glycolysis, two molecules of ATP are used and four molecules are generated, leaving a gain of two molecules of ATP for every molecule of glucose fermented. Let us now look at what happens when glucose is made available to obligate aerobes, facultative anaerobes, and obligate anaerobes.

1. Obligate aerobic cell. Glucose is oxidized to pyruvic acid, but the latter does not remain as the final electron acceptor in the cell. Instead, pyruvic acid is completely oxidized to carbon dioxide and water in a process called *aerobic respiration* (discussed later in this chapter). There are some microorganisms that use inorganic molecules other than oxygen, such as nitrate (NO_3) or sulfate (SO_4), as final electron acceptors. They will be discussed later.
2. Facultative anaerobic cell. The facultative anaerobe in the presence of oxygen completely oxidizes the organic molecule to carbon dioxide and water. When oxygen is absent, the organic molecule is incompletely oxidized in the fermentation process to other organic molecules such as lactic acid.
3. Obligate anaerobic cell. Fermentation is one mechanism used by obligate anaerobes to obtain energy—the other is anaerobic respiration. The fermentation product, pyruvic acid, is but one of many incompletely oxidized products. Various end products are released during fermentation, depending on the organism, the pH of the growth medium, and other factors (Fig. 6-4). These fermentation end products can be useful in the identification of certain microbial species. Many obligate anaerobes produce a distinctive profile of organic acids that can be identified in the clinical laboratory by gas chromatography for species differentiation.

Other Pathways of Glucose Catabolism

In addition to the EMP pathway other pathways of glucose catabolism are available to most microorganisms. They include the hexose monophosphate (HMP) shunt (also called the pentose phosphate cycle) and the Entner-Doudoroff (ED) pathway.

HEXOSE MONOPHOSPHATE SHUNT. The HMP shunt is a complicated series of reactions in which glucose is first converted to glucose 6-phosphate as in the EMP pathway. The HMP shunt then departs from the EMP pathway by oxidation of glucose 6-phosphate to ribulose 5-phosphate, a five-carbon sugar (see Fig. 9-1).

$$\text{Glucose 6-phosphate} \xrightarrow{\text{NADP}} \text{6-phosphogluconate} + NADPH_2$$

$$\text{6-phosphogluconate} \xrightarrow{\text{NADP}} \text{ribulose 5-phosphate} + NADPH_2$$

(NADP = nicotinamide-adenine dinucleotide phosphate; $NADPH_2$ = reduced form of NADP.) Ribulose 5-phosphate is the starting material for the formation of a number of three-, four-, five-, six-, and seven-carbon sugars from which pyruvate can be produced and energy released using a variety of cytoplasmic enzymes. The HMP shunt can be used

Fig. 6-4. Products resulting from the metabolism of pyruvate. The products generated and their concentration vary from one species to another and are influenced by nutritional as well as environmental factors. The end products are enclosed in boxes. (CoA = coenzyme A.)

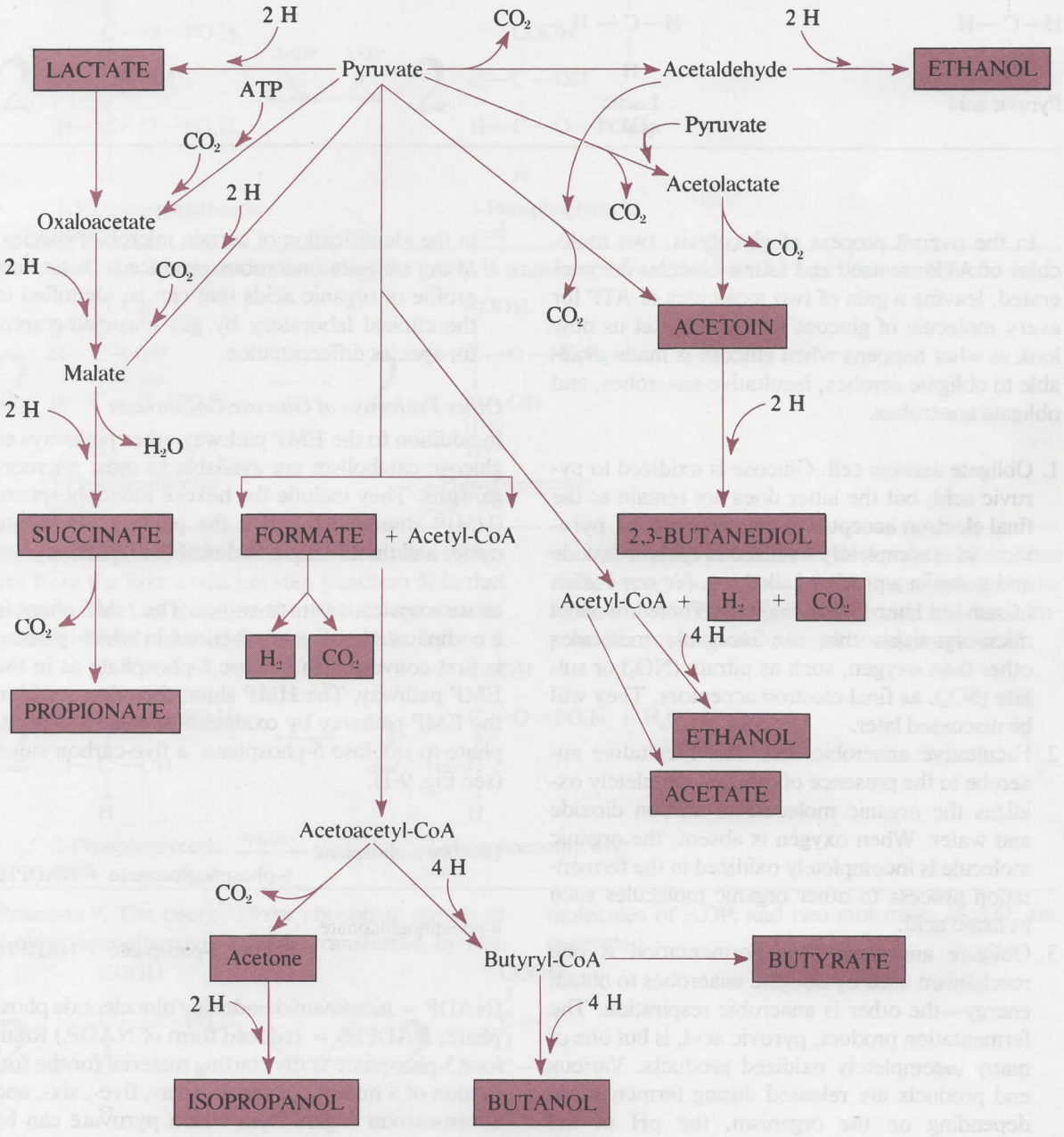
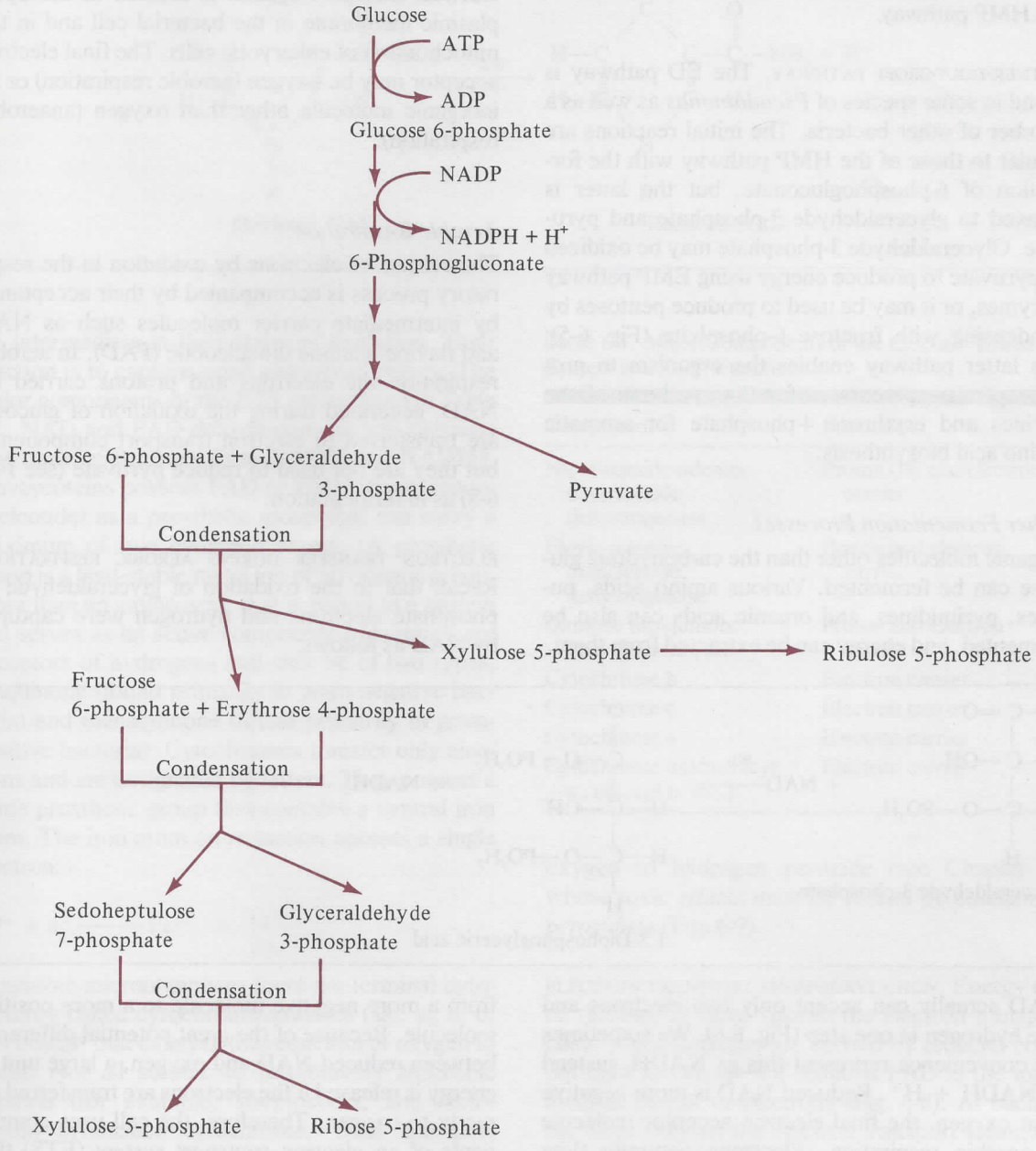


Fig. 6-5. The Entner-Doudoroff pathway for the catabolism of glucose. Note that glyceraldehyde 3-phosphate and pyruvate are produced as in glycolysis, but through a variety of condensations other products such as four-carbon (erythrose), five-carbon (xylulose, ribulose, ribose), and seven-carbon (sedoheptulose) sugars are produced. These sugars can be used in the biosynthesis of other molecules such as nucleic acids, or they may be used in cytoplasmic membrane or cell wall components.



by aerobic as well as anaerobic bacterial species. It is particularly important because one of its main purposes is to supply reducing power in the form of $NADPH_2$ for biosynthesis. The pathway is also a supplier of pentose sugars for nucleic acid synthesis. See Chapter 9 for a more detailed discussion of the HMP pathway.

ENTNER-DOUDOROFF PATHWAY. The ED pathway is found in some species of *Pseudomonas* as well as a number of other bacteria. The initial reactions are similar to those of the HMP pathway with the formation of 6-phosphogluconate, but the latter is cleaved to glyceraldehyde 3-phosphate and pyruvate. Glyceraldehyde 3-phosphate may be oxidized to pyruvate to produce energy using EMP pathway enzymes, or it may be used to produce pentoses by condensing with fructose 6-phosphate (Fig. 6-5); this latter pathway enables the organism to produce pentose precursors for the synthesis of the purines and erythrose 4-phosphate for aromatic amino acid biosynthesis.

Other Fermentation Processes

Organic molecules other than the carbohydrate glucose can be fermented. Various amino acids, purines, pyrimidines, and organic acids can also be fermented, and energy can be extracted from them.

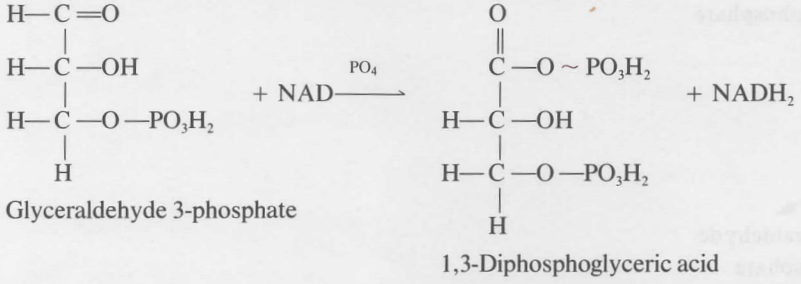
RESPIRATION

Respiration is a cellular energy-generating system in which the electrons released by oxidation are transferred to an electron transport system. The electron transport system is located in the cytoplasmic membrane in the bacterial cell and in the mitochondria of eukaryotic cells. The final electron acceptor may be oxygen (aerobic respiration) or an inorganic molecule other than oxygen (anaerobic respiration).

Aerobic Respiration

The release of electrons by oxidation in the respiratory process is accompanied by their acceptance by intermediate carrier molecules such as NAD and flavine-adenine dinucleotide (FAD). In aerobic respiration the electrons and protons carried by NAD, generated during the oxidation of glucose, are transferred to electron transport components, but they are not used to reduce pyruvate (see Fig. 6-8) as in fermentation.

ELECTRON TRANSFER DURING AEROBIC RESPIRATION. Recall that in the oxidation of glyceraldehyde 3-phosphate electrons and hydrogen were captured by NAD as follows:

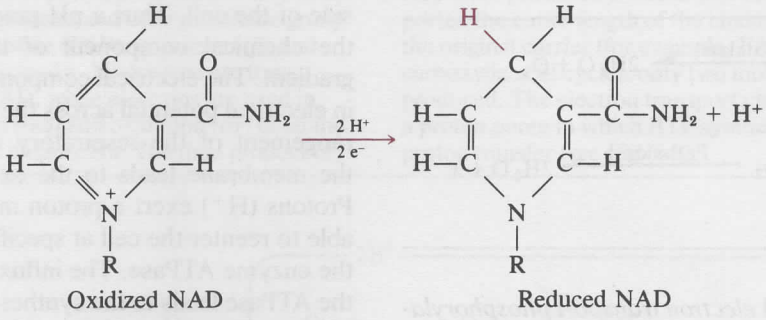


Glyceraldehyde 3-phosphate

1,3-Diphosphoglyceric acid

NAD actually can accept only two electrons and one hydrogen in one step (Fig. 6-6). We sometimes for convenience represent this as $NADH_2$ instead of $NADH + H^+$. Reduced NAD is more negative than oxygen, the final electron acceptor molecule in aerobic respiration. Electrons naturally flow from a more negative molecule to a more positive molecule. Because of the great potential difference between reduced NAD and oxygen, a large unit of energy is released if the electrons are transferred directly to oxygen. Therefore, the cell uses components of an *electron transport system* (ETS) that

Fig. 6-6. Oxidized and reduced forms of nicotinamide-adenine dinucleotide (NAD). The R group is the dinucleotide portion of NAD.



are intermediate in their states of oxidation. Their function is to capture small aliquots of energy. The major components of the ETS are outlined in Table 6-1. NAD and FAD dehydrogenases are enzymes that transfer electrons from NADH and NADPH. Flavoproteins possess FAD or FMN (flavin mononucleotide) as a prosthetic group that can carry a maximum of two hydrogen atoms. (A prosthetic group is a heat-stable metal ion or an organic group, other than an amino acid, that is bound to a protein and serves as its active component.) Quinones are acceptors of hydrogens and may be of two types, ubiquinone (found primarily in gram-negative bacteria) and menaquinone (found primarily in gram-positive bacteria). Cytochromes transfer only electrons and are designated by letters. They possess a heme prosthetic group that contains a central iron atom. The iron atom on reduction accepts a single electron:

$$Fe^{3+} + e^- \longrightarrow Fe^{2+}$$

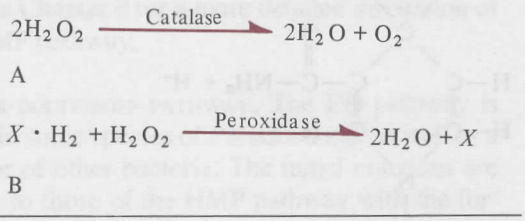
In aerobic microorganisms there are terminal cytochromes called cytochrome oxidases (aa_3, a_3, o, d, and others) that catalyze the reduction of oxygen to water. Not all aerobic or facultatively anaerobic bacteria (for example, streptococci, and lactobacilli) synthesize cytochrome. Their transport chains consist of an oxidase that reduces molecular oxygen to hydrogen peroxide (see Chapter 7), whose toxic effects must be curbed by *catalase* or *peroxidase* (Fig. 6-7).

Table 6-1. Major Components of the Electron Transport System and Their Functions

Component	Function
Nicotinamide-adenine dinucleotide dehydrogenase	Proton (H) and electron carrier
Flavin-adenine dinucleotide dehydrogenase	Proton and electron carrier
Quinone (ubiquinone, menaquinone, etc.)	Proton and electron carrier
Cytochrome b	Electron carrier
Cytochrome c	Electron carrier
Cytochrome a	Electron carrier
Cytochrome oxidase (cyt a_3, aa_3, cyt o, etc.)	Electron carrier

ELECTRON TRANSPORT PHOSPHORYLATION. Energy can be released at three sites in the electron transport chain when electrons are carried by reduced NAD but only at two sites if reduced FAD is the intermediate carrier of electrons (Fig. 6-8). At each of the three sites on the electron transport chain, energy is released and used to synthesize ATP in a

Fig. 6-7. Cellular reactions involved in the metabolism of hydrogen peroxide (H_2O_2). A. Catalase enzyme. B. Peroxidase enzyme. ($X \cdot H_2$ = any reduced organic compound.)



process now called *electron transport phosphorylation*[2]:

$$ADP + PO_4 + \text{energy} \longrightarrow ATP$$

In aerobic respiration the transfer of a pair of electrons from $NADH_2$ to oxygen results in the following products:

$$NADH_2 + 3\ ADP + \tfrac{1}{2}\ O_2 + 3\ PO_4 \longrightarrow NAD + 3\ ATP + H_2O$$

COUPLING OF ATP FORMATION TO ELECTRON TRANSPORT. How ATP synthesis is coupled to electron transport was a subject of debate for many years. In 1963 Peter Mitchell proposed a theory to explain this phenomenon and called it the *chemiosmotic theory*. Experimental studies since 1963 have confirmed this theory, which has been applied not only to respiration but to other cellular processes that involve bioenergetics.

The chemiosmotic theory is based on the notion that the respiratory components span the cytoplasmic membrane and that metabolic energy is conserved and transduced at the bacterial cytoplasmic membrane (or mitochondrial membrane in eukaryotes) in the form of an electrochemical gradient. According to this model, electrons transported down the respiratory chain release free energy that is used to pump protons (H^+) from inside the cell to the outside of the cytoplasmic membrane (Fig. 6-9). When this occurs there is a difference in the H^+ concentration between the inside and outside of the cell. Thus a pH gradient is produced—the chemical component of the electrochemical gradient. The electrical component is the difference in electrical potential across the membrane. The arrangement of the respiratory components across the membrane leads to the extrusion of protons. Protons (H^+) exert a proton motive force and are able to reenter the cell at specific sites that contain the enzyme ATPase. The influx of protons through the ATPase leads to the synthesis of ATP. In respiring cells three protons are translocated for every ATP molecule synthesized. If there is no respiration and ATP is synthesized by substrate phosphorylation, ATP is hydrolyzed by the ATPase system, which now extrudes protons.

The proton motive force provides energy to drive the rotation of flagella, to effect DNA uptake during transformation, and to effect transport of solutes into the cell.

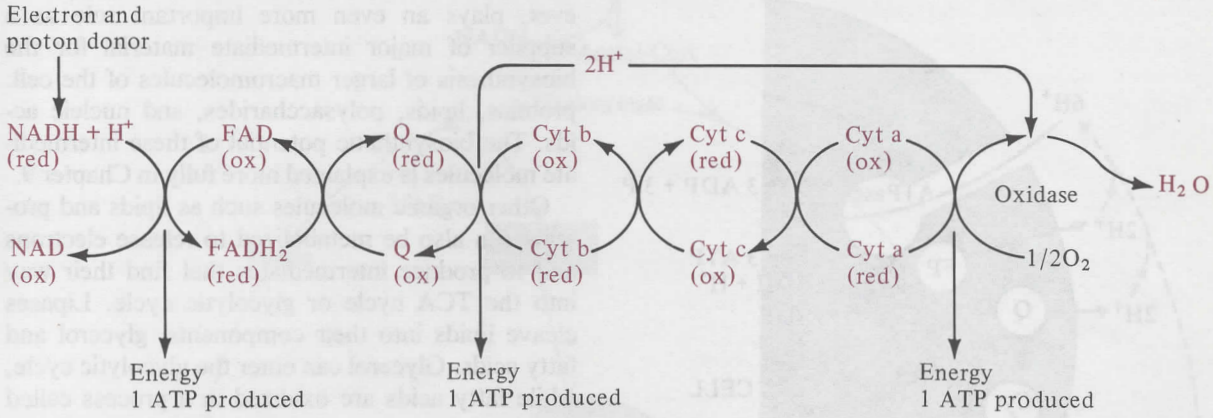
MAJOR SOURCES OF ELECTRONS. We have seen that the transport of a pair of electrons down the respiratory chain results in the formation of three ATP molecules. The source of these electrons is highly reduced organic molecules that can be completely oxidized. For example, when glucose is completely oxidized to CO_2 and H_2O, 12 pairs of electrons can be made available for transport in the ETS.

The formation of H_2O in the ETS has already been explained. The formation of CO_2 as a final oxidation product and the release of electrons take place in a series of reactions called the *tricarboxylic acid (TCA)*, or *Krebs, cycle*. The sequence of reactions is diagrammed in Figure 6-10. Some of the key intermediates in this cycle and the reactions in which they are involved are described here:

1. Pyruvate, a three-carbon unit, in the presence of coenzyme A is oxidized to a two-carbon unit, acetyl-CoA, plus CO_2, with the concomitant reduction of NAD to $NADH_2$. Although this reaction is not actually part of the TCA cycle, it does

[2]*Electron transport phosphorylation* is replacing the older term, *oxidative phosphorylation*, because the process also occurs under anaerobic conditions (oxygen is not involved).

Fig. 6-8. Electron transport chain. NAD accepts electrons and protons from oxidized substrates and transfers them to the electron transport components found in the cytoplasmic membrane of prokaryotic cells. These components include flavoproteins and their prosthetic group flavin-adenine dinucleotide (*FAD*), quinones (*Q*), and cytochromes (*Cyt*) b, c, and a. Hydrogens are transferred only as far as FAD, while electrons are used in the alternate reduction (*red*) and oxidation (*ox*) of all the respiratory transport components. The final product of oxidation is water. Energy is released in sufficient concentration at three places in the transport chain to produce a molecule of adenosine triphosphate (*ATP*). Three ATP's are produced for each pair of electrons transported the entire length of the chain. If reduced FAD is the original carrier (for example, FAD is used in the tricarboxylic acid cycle), only two molecules of ATP are produced. The electron transport chain is believed to be a proton pump in which ATP synthesis is coupled to proton transfer (see Fig. 6-9).



supply acetyl-CoA, an important component of the cycle.

2. Acetyl-CoA condenses with a four-carbon unit, oxaloacetate, to form the six-carbon citrate.
3. Citrate undergoes a series of reactions to form isocitrate. Isocitrate is oxidized to alpha-ketoglutarate with the release of a molecule of CO_2 and the reduction of NAD to $NADH_2$.
4. alpha-Ketoglutarate is further oxidized in the presence of CoA to succinyl-CoA with the liberation of one molecule of CO_2 and the reduction of NAD to $NADH_2$.
5. Succinyl-CoA loses the CoA moiety, and energy is trapped by guanosine diphosphate (GDP) to form guanosine triphosphate (GTP). ATP is later produced from the reaction.

$$GTP + ADP \longrightarrow GDP + ATP$$

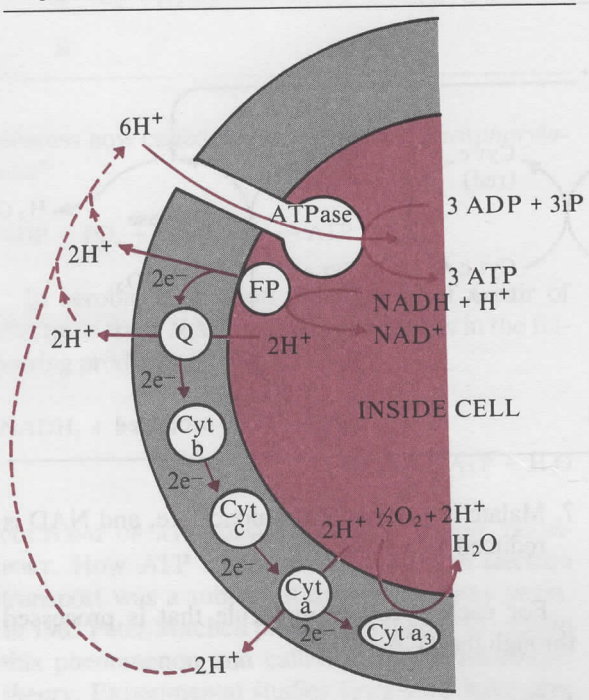
6. Succinate is oxidized to fumarate, and FAD is reduced to $FADH_2$.
7. Malate is oxidized to oxaloacetate, and NAD is reduced to $NADH_2$.

For each pyruvate molecule that is processed through the TCA cycle

1. Four pairs of electrons are removed and accepted by four molecules of NAD; thus twelve ATP molecules are produced when four molecules of reduced NAD travel through the ETS.
2. One pair of electrons is removed and accepted by FAD; thus two ATP molecules are produced when reduced FAD travels through the ETS.
3. One molecule of ATP is produced in the substrate phosphorylation of GDP to GTP.
4. Two molecules of CO_2 are released in the TCA cycle, and one molecule of CO_2 is released in the oxidation of pyruvate to acetyl-CoA.

It is obvious that, in terms of efficiency, more energy can be obtained from respiration than from

Fig. 6-9. Chemiosmosis—proposed mechanism for coupling of electron transport to ATP production. Protons generated in the cell are transported from inside the cytoplasmic membrane to the exterior, causing the inside of the cell to be alkaline with respect to the outside. Electrons are transported along the respiratory components to oxygen and the formation of water. Protons extruded from the cell can reenter the cell at the sites of ATPase where ATP is produced. (FP = flavoprotein; Q = quinone; Cyt = cytochrome; iP = phosphate.)



fermentation. During aerobic respiration the bacterial cell produces 38 ATP molecules per molecule of glucose oxidized:

2 ATP via substrate phosphorylation in glycolysis
6 ATP via electron transport phosphorylation (reduced NAD produced during glycolysis)
30 ATP via electron transport phosphorylation (oxidation in TCA cycle)

Obligate anaerobes have a net gain of only 2 ATP's via substrate phosphorylation.

Because ATP is a fairly unstable molecule, it is easily hydrolyzed in water to ADP, PO_4, and energy. The energy released is wasted as heat unless it is immediately used in some reaction. To conserve energy for later use, the cell stores it by synthesizing larger macromolecules such as lipids and polysaccharides from which it can later extract the energy needed for biosynthetic reactions.

It is true that considerably more ATP can be produced when glucose is completely oxidized to CO_2 and H_2O during respiration. The TCA cycle, however, plays an even more important role as a supplier of major intermediate material for the biosynthesis of larger macromolecules of the cell: proteins, lipids, polysaccharides, and nucleic acids. The biosynthetic potential of these intermediate molecules is explained more fully in Chapter 9.

Other organic molecules such as lipids and proteins can also be metabolized to release electrons and to produce intermediates that find their way into the TCA cycle or glycolytic cycle. Lipases cleave lipids into their components: glycerol and fatty acids. Glycerol can enter the glycolytic cycle, while fatty acids are oxidized in a process called *beta oxidation*. Beta oxidation brings about the release of two carbon fragments and their conversion to acetyl-CoA. Acetyl-CoA can directly enter the TCA cycle (Fig. 6-11).

Proteins are catabolized by proteases into their component amino acids. Amino acids lose their amino group in a process called *deamination*, and this can result in the formation of organic acids such as pyruvic acid, alpha-ketoglutaric acid, or others that can enter the TCA cycle (Fig. 6-11).

Anaerobic Respiration

Respiration does not always involve oxygen as the final electron acceptor. Alternative electron acceptors such as nitrate (NO_3), sulfate (SO_4), carbonate, or fumarate can be used by some bacterial species, especially the soil microorganisms. When the electron acceptor in respiration is an inorganic molecule other than oxygen or an organic molecule, the process is called *anaerobic respiration*.

In species pathogenic to humans, nitrate and fumarate are the primary electron acceptors, with nitrite and succinate, respectively, as the final prod-

Fig. 6-10. The tricarboxylic acid (TCA) cycle (also called Krebs or citric acid cycle). The primary end products, carbon dioxide and four pairs of electrons, are the result of oxidation of pyruvate and are shown in boxes. The hydrogens are picked up by carriers such as NAD and FAD and transported to the electron transport system (*ETS*). The conversion of pyruvate to acetyl-CoA is not part of the TCA cycle, but the hydrogens released in the oxidation can also be carried to the ETS. (CoA = coenzyme A; GTP = guanosine triphosphate; GDP = guanosine diphosphate.)

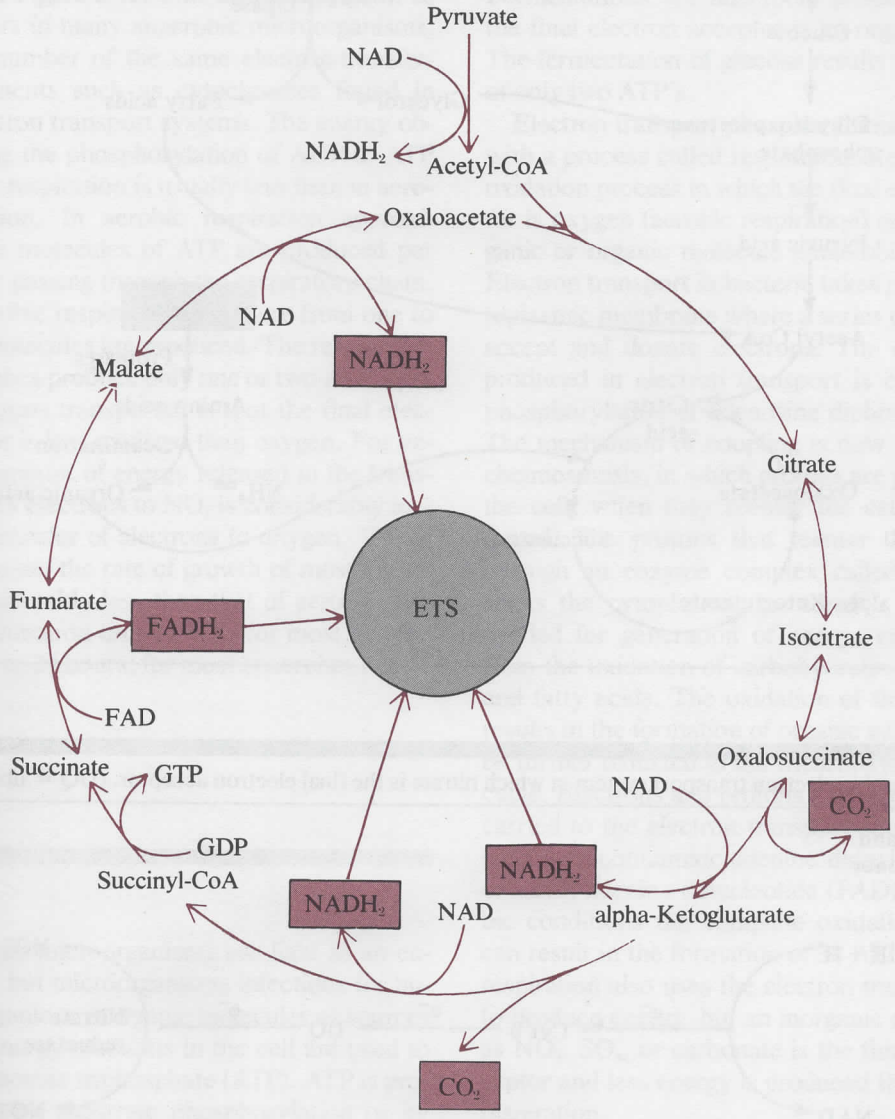


Fig. 6-11. Mechanism by which energy sources such as polysaccharides, lipids, and proteins can be metabolized to products that enter the tricarboxylic acid cycle.

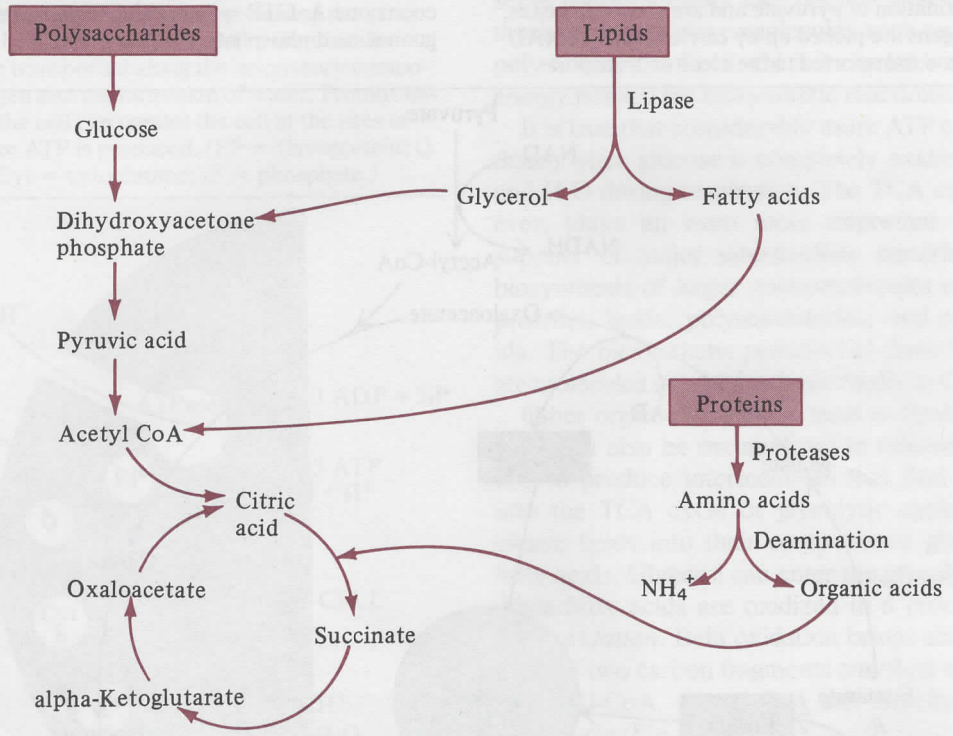
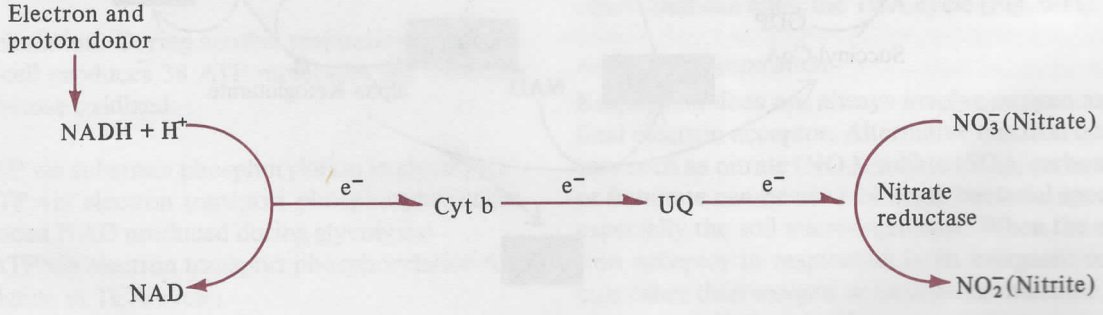


Fig. 6-12. Anaerobic electron transport system in which nitrate is the final electron acceptor. (UQ = ubiquinone.)



ucts of reduction. Nitrate is the more common final electron acceptor, and *nitrate reductase* is the enzyme that catalyzes this reduction. The enzyme is induced in the absence of oxygen and repressed in the presence of oxygen. An anaerobic ETS flow chart with nitrate as the final electron acceptor is illustrated in Figure 6-12. This electron system, as well as others in many anaerobic microorganisms, contains a number of the same electron-transferring components such as cytochromes found in aerobic electron transport systems. The energy obtained during the phosphorylation of ADP to ATP in anaerobic respiration is usually less than in aerobic respiration. In aerobic respiration approximately three molecules of ATP are produced per electron pair passing through the respiratory chain, but in anaerobic respiration anywhere from one to three ATP molecules are produced. The reason that some anaerobes produce only one or two ATP's per pair of electrons transported is that the final electron acceptor is less oxidized than oxygen. For example, the amount of energy released in the transfer of a pair of electrons to NO_3 is considerably less than in the transfer of electrons to oxygen. This is the major reason the rate of growth of most anaerobes is considerably less than that of aerobic species. The incubation time at 37°C for most aerobic species is 18 to 24 hours; for most anaerobes it is 48 to 72 hours.

SUMMARY

Photosynthetic microorganisms use light as an energy source, but microorganisms infectious for humans use organic or inorganic molecules as sources of energy. Energy reactions in the cell are used to produce adenosine triphosphate (ATP). ATP is produced either by substrate phosphorylation or by electron transport phosphorylation. Substrate phosphorylation takes place in a process called glycolysis, a type of fermentation in which glucose is converted to pyruvic acid. During the oxidation process in glycolysis, energy is trapped in a substrate molecule before being used to produce ATP. Fermentations are anaerobic processes in which the final electron acceptor is an organic molecule. The fermentation of glucose results in the net gain of only two ATP's.

Electron transport phosphorylation is associated with a process called respiration. Respiration is an oxidation process in which the final electron acceptor is oxygen (aerobic respiration) or another inorganic or organic molecule (anaerobic respiration). Electron transport in bacteria takes place in the cytoplasmic membrane where a series of components accept and donate electrons. The energy that is produced in electron transport is coupled to the phosphorylation of adenosine diphosphate (ADP). The mechanism of coupling is now believed to be chemiosmosis, in which protons are pumped out of the cell; when they reenter the cell ATP is produced. The protons that reenter the cell do so through an enzyme complex called ATPase that spans the cytoplasmic membrane. The electrons needed for generation of energy can be derived from the oxidation of carbohydrates, amino acids, and fatty acids. The oxidation of these molecules results in the formation of organic acids, which can be further oxidized in the tricarboxylic acid (TCA) cycle. Electrons and protons that are generated are carried to the electron transport chain by carriers such as nicotinamide-adenine dinucleotide (NAD) or flavin-adenine dinucleotide (FAD). Under aerobic conditions the complete oxidation of glucose can result in the formation of 38 ATP's. Anaerobic respiration also uses the electron transport system to produce energy, but an inorganic molecule such as NO_3, SO_4, or carbonate is the final electron acceptor and less energy is produced than by aerobic respiration.

QUESTIONS FOR STUDY

1. What are the differences between fermentation and respiration?
2. Why do electrons naturally flow from reduced NAD to an acceptor molecule such as oxygen?
3. What are the principal electron and proton carriers in the cell (not those in the electron transport chain)?
4. What inorganic molecules can serve as final electron acceptors in anaerobic respiration?
5. Write out the two reactions in glycolysis in which ATP is produced.
6. What happens to reduced NAD during fermentation?
7. Why is glycolysis considered an incomplete oxidation process?
8. Can oxidations occur in the absence of oxygen? Explain.
9. What is the principal function of the TCA cycle?
10. How is ATP produced according to the chemiosmotic theory?
11. What is the cytochrome called that is involved in the reduction of oxygen to water?
12. Why is the electron transport chain needed by the cell?
13. List some important products of glucose fermentation.
14. How can the cell utilize the TCA cycle if its energy source is fatty acids?
15. Explain the difference between substrate phosphorylation and electron transport phosphorylation.

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7. NUTRITION, CULTIVATION, AND ISOLATION OF BACTERIA

OBJECTIVES

To list the elements required by all cells and the inorganic and organic forms in which they appear in the environment

To define the oxygen requirements of obligate aerobes, microaerophiles, facultative anaerobes, and obligate anaerobes

To list the detrimental products of oxygen metabolism and how the cell removes them

To explain the different mechanisms used by the cell to transport nutrients across the cytoplasmic membrane

To briefly explain the function of: all-purpose, enriched, selective, differential, enrichment, and transport media in the cultivation of bacteria

To describe the methods used to obtain a pure culture of bacteria

To list those methods that are used to preserve isolated species of bacteria

To list some of the important minerals required by microorganisms and to describe their function

To define: nutrient, autotroph, heterotroph, nitrogen fixation, superoxide, synthetic medium, halophilic, GasPak, axenic, beta hemolysis, alpha hemolysis

Nutrition
- Chemical Requirements
  - Water
  - Carbon
  - Nitrogen
  - Oxygen
    - Obligate Aerobes
    - Microaerophiles
    - Facultative Anaerobes
    - Obligate Anaerobes
    - Products of Oxygen Metabolism
  - Sulfur
  - Phosphorous
  - Minerals
  - Organic Growth Factors
- Nutritional Classification
- Nutrient Transport

Laboratory Cultivation
- Types of Culture Media
  - Media Based on Chemical Content
    - Synthetic or Defined
    - Nonsynthetic
  - Media Based on Laboratory Use
    - All-Purpose Media
    - Enriched Media
    - Selective Media
    - Differential Media
    - Enrichment Media
    - Transport Media
  - Factors in the Preparation of a Culture Medium
    - Fluidity
    - pH
    - Sterilization
    - Oxygen Tension
      - Isolation of Aerobes
      - Isolation of Anaerobes
    - Temperature

Laboratory Isolation
- Methods for Isolating Bacteria
  - Streak Plate
  - Pour Plate
  - Membrane Filter
- Media Used for Primary Isolation
- Preservation of Isolated Microorganisms
  - Subculture
  - Metabolic Reduction
  - Drying
  - Freezing
  - Freeze-Drying (Lyophilization)

NUTRITION

Nutrition is a process that is concerned with the chemical factors necessary for the normal growth and development of an organism. The chemicals that satisfy these requirements are called *nutrients*. The bacteria are a very diverse group in terms of their chemical requirements, which may be totally inorganic, organic, or a mixture of both. In some bacteria the chemical requirements are minimal: A simple inorganic salt solution plus a carbon source may be all that is needed for growth. In other bacteria the chemical requirements may include several amino acids, vitamins, and purine or pyrimidine bases. In general the pathogenic organisms or parasites have more complex needs than those whose natural habitat is outside the animal host. Parasites, having relied on the nutrients of their host for so long a time, have apparently lost some of their biosynthetic capabilities.

The elements essential to bacteria are no different from those essential to most living organisms. Some of the elements are the major building blocks of organic material: carbon, nitrogen, sulfur, and phosphorous. Other elements are not part of the building block material but are nevertheless required for growth. Many of these minor nutrients are minerals such as calcium, potassium, and others. The purpose of this chapter is to outline the

major and minor nutrients required for growth and to discuss how various nutrients are used in the isolation and cultivation of microorganisms.

CHEMICAL REQUIREMENTS

Water

Water is a universal solvent that quantitatively makes up the major portion of all cells. It is used to hydrate various substances, which allows them to be transported across the cytoplasmic membrane into the cytoplasm of the cell. Water also participates in many biochemical reactions in the cell. It is required for some reactions (hydrolysis) and is formed as an end product in others.

Carbon

Carbon can be used by the cell in various inorganic or organic forms. Inorganic carbon may exist as carbon monoxide (CO), carbon dioxide (CO_2), and carbonate (CO_3). Organic forms of carbon include the major polymers—proteins, carbohydrates, lipids, and nucleic acids—as well as the various monomers of which they are composed (see Chapter 2).

Carbon is the backbone of all cellular material, and it may be derived from inorganic or organic sources. Inorganic carbon as CO_2 is used in large quantities by photosynthetic organisms for incorporation into cellular organic molecules. Organisms that use CO_2 as a source of carbon are called *autotrophs*. There are microorganisms that also use carbon dioxide as a terminal acceptor of electrons in respiration. Most microorganisms, however, use carbon in its organic form. These organic carbon compounds are used in biosynthetic reactions to produce other organic molecules in the cell, and they may also be broken down to supply energy to the cell. Depending on the species, the carbon requirements of a cell may be satisfied by simple two-carbon compounds such as acetate (CH_3COO^-) or by more complex molecules such as glucose ($C_6H_{12}O_6$). Organisms using organic molecules as a source of carbon are called *heterotrophs*.

Microorganisms have the enzymatic machinery to oxidize many types of organic molecules. These enzymes may be intracellular and used for breaking down organic molecules transported into the cytoplasm, or they may be extracellular enzymes used to hydrolyze substances outside the cell that are too large to enter the cell in an unaltered form.

Nitrogen

Nitrogen is found in many organic molecules and is also available in various inorganic forms. Inorganic nitrogen as nitrogen gas (N_2), nitrate (NO_3), nitrite (NO_2), or ammonia (NH_3) can be used by a wide variety of microorganisms, depending on the species and its particular habitat. The nitrogen in organic compounds is usually available to the cell in the form of amino acids.

Some microorganisms degrade proteins into their component amino acids and use them for synthesis of cellular proteins. Sometimes the amino group on the amino acid is removed (*deamination*) and the remainder of the molecule is used as precursor material for the synthesis of other organic compounds. Inorganic forms of nitrogen are usually reduced to NH_3, which is assimilated by the cell into an organic form. An important assimilation process is the incorporation of NH_3 into the glutamate molecule to produce glutamine (Fig. 7-1). Glutamine can therefore store nitrogen and serve as a source of amino groups as they are needed by the cell; for example, some amino sugars are required in the cell wall.

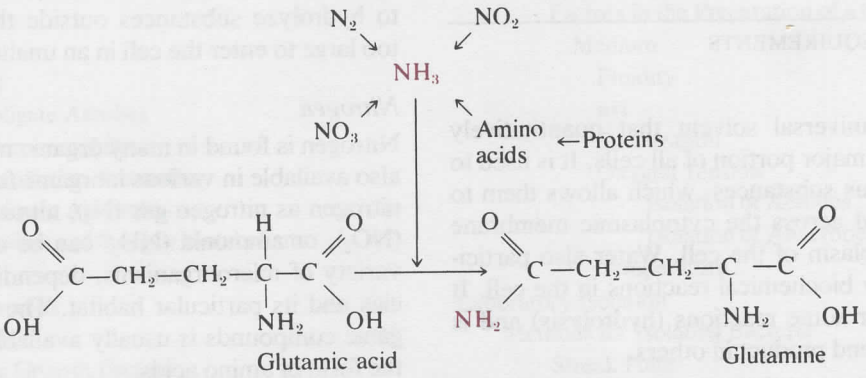
Nitrate is a particularly common inorganic form of nitrogen that is reduced to NH_3 through the activity of two enzymes:

$$NO_3 \xrightarrow[\text{reductase}]{\text{Nitrate}} NO_2 \xrightarrow[\text{reductase}]{\text{Nitrite}} NH_3$$

Nitrate can also serve in a dissimilatory role, that is, it is used as a terminal electron acceptor in anaerobic respiration and is not assimilated into organic matter in the cell. Anaerobic respiration is discussed in Chapter 6.

Nitrogen gas can be used as a source of nitrogen by a variety of microorganisms, many of which are

Fig. 7-1. The flow of nitrogen from inorganic to organic forms within the cell. Nitrogen sources available to the cell include inorganic or organic forms, and both are converted to NH_3. NH_3 can be assimilated into an organic form by the amination of glutamic acid, which results in the formation of glutamine.



associated with aquatic and soil environments; there are, however, a few genera of infectious bacteria such as *Klebsiella, Enterobacter, Clostridium*, and *Citrobacter* that can use nitrogen gas. The use of nitrogen gas by these microorganisms is called *nitrogen fixation*. This process, which is catalyzed by the enzyme nitrogenase, requires tremendous amounts of energy and takes place in the absence of oxygen. The overall reaction is

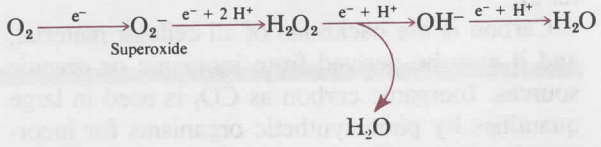
$$6\,H^+ + 6\,e^- + N{\equiv}N\ (\text{nitrogen gas}) \xrightarrow{\text{Nitrogenase}} 2\,NH_3$$

One of the important aspects of this process for medical personnel is that microorganisms contaminating enclosed fluids and devoid of soluble nitrogen compounds may be able to grow because of their ability to fix nitrogen. Some hospital-associated infections have been traced to this microbial property.

Oxygen

Air at one atmosphere consists primarily of nitrogen (78 percent), oxygen (20 percent), and carbon dioxide (0.03 percent). Gaseous nitrogen can be used by only a few species of bacteria, the nitrogen-fixing bacteria. Carbon dioxide as a sole carbon source can be used by only the autotrophic bacteria. Oxygen, however, plays a major role in the metabolism of most organisms because it functions in respiration or energy production. According to their gaseous requirements, microbes are classified as *obligate aerobes, microaerophiles, obligate anaerobes*, and *facultative anaerobes*.

Fig. 7-2. The sequential reduction of oxygen in the cell, which can result in the formation of water but can also produce toxic intermediates such as superoxide, hydrogen peroxide, and hydroxide radical. Each reaction represents a univalent reduction involving a single electron.



OBLIGATE AEROBES. Obligate aerobes are totally dependent on the presence of oxygen and are unable to grow in its absence. The oxygen requirement for obligate aerobes is usually expressed as one atmosphere or 20 percent. The use of oxygen as the terminal electron acceptor by these organisms does

not always mean that they will grow faster than anaerobes. *Mycobacterium tuberculosis*, for example, is an obligate aerobe that has one of the longest generation times of any bacterial species (a visible colony may not appear within a week when inoculated on an agar surface).

MICROAEROPHILES. Microaerophiles require oxygen but only at concentrations less than one atmosphere (usually around 0.2 atmospheres). High concentrations of oxygen may not be lethal to these organisms, but they do inhibit growth. Many microaerophiles grow best at elevated concentrations of carbon dioxide, and they are sometimes referred to as *capneic*.

FACULTATIVE ANAEROBES. Facultative anaerobes are capable of growth in the presence of oxygen or in its absence. They grow best when oxygen is present in the environment and they can use the respiratory pathway but in the absence of oxygen are forced to use fermentative pathways.

OBLIGATE ANAEROBES. A wide variety of microorganisms found in the soil as well as in mammalian hosts are obligate anaerobes and cannot use oxygen. Two of the most important genera belonging to this group are *Clostridium* and *Bacteroides*. The sensitivity of this group to oxygen varies. Some lack a cytochrome system and oxygen is not reduced; others reduce oxygen to toxic compounds that are deleterious to the cell.

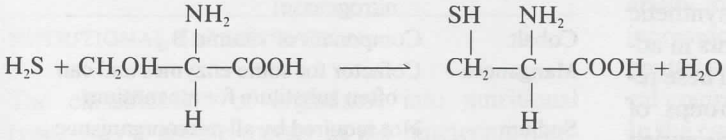
PRODUCTS OF OXYGEN METABOLISM. We have seen in Chapter 6 that oxygen as a final electron acceptor is reduced to water by cytochrome oxidase. This type of reduction is tetravalent, that is, four electrons are transferred (Fig. 7-2), but not all of the oxygen is converted to water. Some microorganisms in addition to cytochrome oxidase also possess flavoprotein oxidases that reduce oxygen to *hydrogen peroxide*, a divalent reduction. This type of oxidation poses no serious threat to the microorganism because they also produce catalase or peroxidase, each of which breaks down hydrogen peroxide (see Fig. 6-7). The tetravalent reduction of oxygen occurs in four successive univalent steps. Univalent reduction of oxygen results in the formation of *superoxide* anion O_2^-, which is highly toxic. It is very likely that an electron transport system unable to handle excess amounts of oxygen could convert some of it to the superoxide anion. Superoxide can be made harmless if the microbial cell produces *superoxide dismutase*, which catalyzes the following reaction:

$$O_2^- + O_2^- \xrightarrow[\text{dismutase}]{\text{Superoxide}} H_2O_2 + O_2$$

Superoxide dismutase has been found in all aerobes so far examined and appears in varying quantities in some aerotolerant anaerobes. *Aerotolerant* anaerobes cannot grow in the presence of oxygen, but oxygen is not lethal to them.

Sulfur

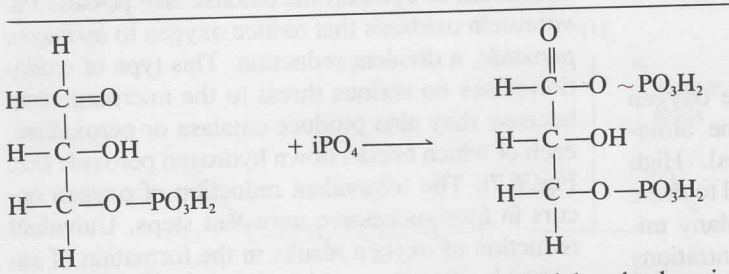
Sulfur is an important constituent of the amino acids cysteine and methionine and also exists in an inorganic form as sulfate (SO_4). Sulfur is assimilated and reduced to hydrogen sulfide (H_2S) and then incorporated into an organic phase according to the following reaction:



Hydrogen sulfide Serine Cysteine

Phosphorous

Phosphorous plays an important part in nucleic acid metabolism. Inorganic phosphate (iPO_4) is the source of phosphorous most widely available to the cell. Inorganic phosphate can become part of the organic phase of the cell only during the process of glycolysis.



Glyceraldehyde 3-phosphate

1,3-Diphosphoglyceric acid

The phosphate eventually becomes part of the ATP (adenosine triphosphate) molecule. ATP is used either in energy-requiring reactions or as one of the nucleoside triphosphates required in nucleic acid biosynthesis.

Minerals

Mineral requirements of microorganisms vary considerably. Some minerals, such as calcium, magnesium, potassium, and iron, are needed by practically all bacteria. Other minerals, such as sodium, zinc, molybdenum, cobalt, copper, and manganese, are usually required in small or trace amounts. In most instances it is not necessary to add trace elements to laboratory media since many are normal contaminants of tap or even "distilled" water. The more important minerals and their functions in microbial metabolism are summarized in Table 7-1. Iron plays a very important role in the infectious disease process; this is discussed in detail in Chapter 12.

Organic Growth Factors

Some microbes, because of their poor biosynthetic properties, need special organic components in addition to their usual growth requirements. These requirements can be divided into three groups of compounds: vitamins, amino acids, and the purine or pyrimidine bases. The vitamins are important

Table 7-1. Important Minerals and Their Functions in Microbial Metabolism

Element	Function
Magnesium	Stabilization of nucleic acids and ribosomes; required in some enzymatic reactions
Potassium	Some enzymatic reactions dependent on its presence; but other monovalent cations can substitute for it; especially important in reactions involved in protein synthesis
Iron	Important component of the cytochromes; involved in diphtheria toxin production; important in bacterial disease
Calcium	Cofactor for some enzymes; required for attachment of some bacteriophages to *Escherichia coli*; important for spore formation
Zinc	Cofactor for some enzymes
Molybdenum	Primary function in nitrogen metabolism, particularly nitrogen fixation (a component of the enzyme nitrogenase)
Cobalt	Component of vitamin B_{12}
Manganese	Cofactor for some enzymes and can often substitute for magnesium
Sodium	Not required by all microorganisms; most marine microorganisms require it

Table 7-2. Vitamins and Their Functions in Microbial Metabolism

Vitamin	Function
Thiamine	As thiamine pyrophosphate, a coenzyme involved in decarboxylation reactions
Riboflavin	In combined form such as flavin-adenine dinucleotide (FAD), a coenzyme important in oxidation-reduction reactions
Niacin	In combined form such as nicotinamide-adenine dinucleotide (NAD) or its phosphorylated derivative (NADP), a coenzyme in oxidation-reduction reactions
Pantothenic acid	Part of the coenzyme A (CoA) molecule; functions as a carrier of acyl groups
Pyridoxal	In phosphorylated form, functions as a coenzyme; important in amino acid metabolism, particularly group transfer reactions involving the amino group
Biotin	Coenzyme important as a carrier of CO_2
Folic acid	In reduced form (tetrahydrofolic acid), acts as a carrier of formyl groups; especially important in purine biosynthesis
Vitamin B_{12}	Coenzyme functioning as a carrier of alkyl groups

organic components of many enzymes and for this reason are called *coenzymes*. The vitamins and their functions in microbial metabolism are summarized in Table 7-2. All of the growth factors can be added to the growth medium in minute amounts, especially the vitamins, which are added in microgram quantities.

NUTRITIONAL CLASSIFICATION

The classification of organisms into nutritional types was a simple matter in the nineteenth century because few microorganisms had been identified. In this century, however, with our increased knowledge of metabolism and the relationship of microorganisms to their environment, many new nutritional categories have been proposed. The microorganisms are generally separated into nutritional types based on the nature of their carbon and energy sources. This classification can be broken down as follows:

1. If the energy source is light, the organism is called a *phototroph*.
2. If the energy source is chemical, the organism is called a *chemotroph*.
3. If the carbon source is CO_2, the organism is called an *autotroph*.
4. If the carbon source is organic, the organism is called a *heterotroph*.

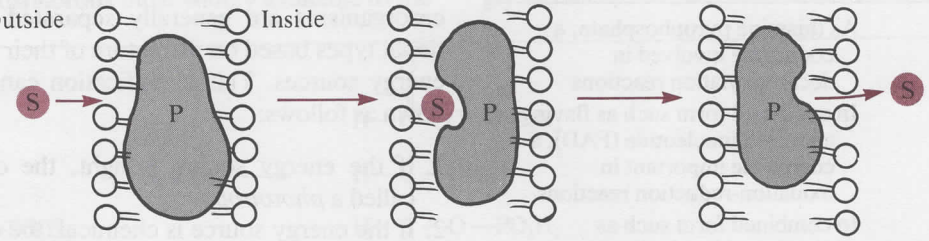
By combining these terms we arrive at the following nutritional types:

1. *Photoautotroph*. Light is the energy source, and CO_2 is the carbon source. Examples are plants and the purple and green sulfur bacteria.
2. *Photoheterotroph*. Light is the energy source, and organic compounds serve as the carbon source. Examples are the photosynthetic nonsulfur bacteria.
3. *Chemoautotroph*. Reduced inorganic compounds are the energy source, and CO_2 is the carbon source. Examples are the iron and hydrogen bacteria.
4. *Chemoheterotroph*. Organic compounds serve as the carbon and energy sources. Most parasitic bacteria fall into this group. Humans and most animals are also part of this group.

NUTRIENT TRANSPORT

Bacteria show a tremendous versatility in their ability to metabolize a large number of organic and inorganic compounds. A bacterial growth medium contains a variety of molecules differing in chemical composition, size, shape, and electrical charge. In the case of large organic molecules such as polysaccharides and proteins that are unable to pene-

Fig. 7-3. Mechanism of facilitated diffusion. A solute (*S*) interacts with transport protein (*P*) in cytoplasmic membrane and is carried across the membrane.



trate the cell membrane, some bacterial cells have the necessary genetic information for the synthesis of enzymes that can be released extracellularly to hydrolyse polymers into simpler molecular units.

Molecules of solute may enter the cell either passively or actively. Passive processes require no energy—the molecule simply moves from an area of high concentration to one of lower concentration until an equilibrium is maintained. Most molecules, because of their size, charge, or hydrophilic or hydrophobic nature, do not ordinarily enter the cell by passive processes. Passive processes, which include osmosis (see Chapter 4), simple diffusion, and facilitated diffusion, are the least used by the microbial cell under most ordinary circumstances. The microbial cell is frequently in environments where the concentration of a solute inside the cell is greater than that outside the cell. In other words, the cell must assimilate nutrients from the environment against a concentration gradient. This type of nutrient movement requires an *active transport* process in which energy is expended. The following are all of the mechanisms used by the cell for nutrient transport:

1. Passive diffusion is the movement of a solute from an area of higher concentration to one of lower concentration. It does not involve a carrier molecule, and energy is not required for the process.
2. Facilitated diffusion is similar to passive diffusion except that a carrier molecule located in the membrane conveys the solute and accelerates the attainment of equilibrium. The carrier is called a permease (Fig. 7-3).
3. Active transport makes use of a carrier molecule to transport the solute, in an unaltered form, across the membrane into the cytoplasm. A source of energy, either chemical or electrical, is required when the solute is transported against a concentration gradient but not if the solute concentration is greater outside the cell. In active transport the solute molecule that appears in the medium remains unchanged during its transfer into the cytoplasm of the cell (Fig. 7-4). Inorganic ions as well as amino acids and sugars are transported by this mechanism.
4. Group translocation is similar to active transport except the transported solute is changed chemically during its passage across the cell membrane. One of the principal mechanisms believed to operate in this process is the *phosphoenolpyruvate sugar phosphotransferase system*. Phosphoenolpyruvate is an intermediate produced in the metabolic process called glycolysis (see Chapter 6) in which glucose is oxidized. Phosphoenolpyruvate contains a high-energy phosphate molecule, which is transferred to the sugar with the participation of some intermediates (Fig. 7-5). Sugars believed to be transferred by this system include glucose, lactose, fructose, mannitol, and sorbitol.

Fig. 7-4. Mechanism of active transport. Solute (*S*) interacts with transport protein (*P*). The interaction requires energy, which is generated by hydrolysis of adenosine triphosphate (ATP) to adenosine diphosphate (ADP).

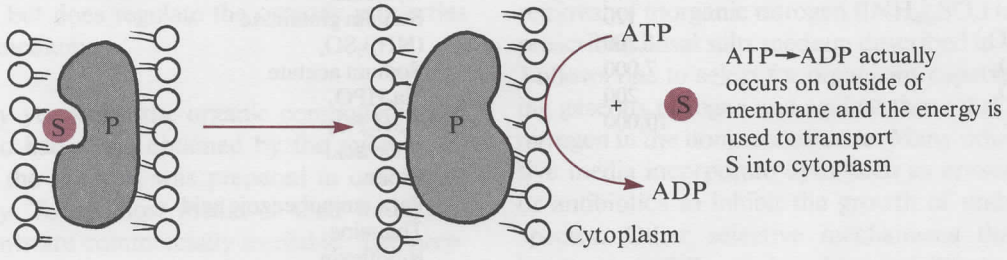


Fig. 7-5. Mechanism of group translocation, the phosphoenolpyruvate sugar phosphotransferase system. A. Phosphate (*P*) is transferred from phosphoenolpyruvate (*PEP*) to enzyme I (*I*). Enzyme I is nonspecific, and up to 10 different sugars may be transported. B. The phosphate on enzyme I (*I*~*P*) is transferred to a heat-stable protein (*HPr*). C. The phosphate on HPr (*HPr*~*P*) is transferred to a sugar-specific component, enzyme III (*III*~*P*). D. The phosphate on enzyme III (*III*~*P*) is transferred to a sugar (*S-P*) and requires enzyme II (*II*), which serves as the permease and translocates the sugar from the outer membrane into the cytoplasm of the cell. (From M. H. Saier, Jr., *Bacteriol. Rev.* 41:856, 1977.)

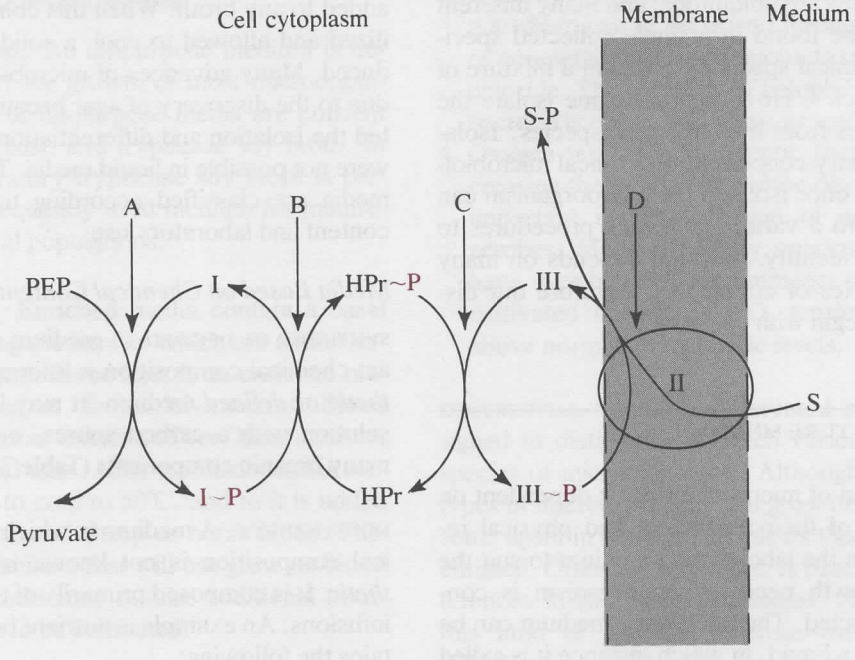


Table 7-3. Chemical Composition of Two Kinds of Synthetic Media[a]

Medium A (mg/liter)		Medium B (mg/liter)	
$CaCl_2$	15	Cysteine	15
$MgSO_4$	120	Sodium glutamate	500
$(NH_4)_2SO_4$	1,200	$(NH_4)_2SO_4$	1,200
Na_2HPO_4	7,000	Sodium acetate	10,000
NaH_2PO_4	200	Na_2HPO_4	7,000
Glucose	10,000	NaH_2PO_4	1,200
		Folic acid	0.005
		Biotin	0.0025
		Para-aminobenzoic acid	0.1
		Thiamine	0.5
		Riboflavin	0.5
		Pyridoxal	1.0
		Pantothenate	0.5
		Nicotinic acid	1.0
		Glucose	10,000

[a]Medium A is a basal salts minimal medium used to cultivate such enteric organisms as *Escherichia coli*. Medium B is a more complex medium used to cultivate some species of oral streptococci.

LABORATORY CULTIVATION

Microorganisms are ubiquitous, and many different species may be found in a single collected specimen. Most clinical specimens contain a mixture of microbial species. How then does one isolate the wanted species from the unwanted species? Isolation is of primary concern to the clinical microbiologist because once isolated the microorganism can be subjected to a variety of testing procedures to determine its identity. Isolation depends on many of the principles of cultivation; therefore our discussion will begin with cultivation.

TYPES OF CULTURE MEDIA

The cultivation of microorganisms is dependent on a knowledge of their nutritional and physical requirements. In the laboratory a medium to suit the particular growth needs of an organism is concocted or selected. The laboratory medium can be in the form of a liquid, in which instance it is called a *broth*, or in the form of a solid, in which case agar is added to the broth. *Agar*, a carbohydrate isolated from seaweed, is not digested by bacteria and does not serve as a nutrient in the medium. Agar is commercially available as fine granules that can be added to any broth. When this combination is sterilized and allowed to cool, a solid medium is produced. Many advances of microbiology have been due to the discovery of agar because it has permitted the isolation and differentiation of bacteria that were not possible in liquid media. Today laboratory media are classified according to their chemical content and laboratory use.

Media Based on Chemical Content

SYNTHETIC OR DEFINED. A medium in which the exact chemical composition is known is called a *synthetic* or *defined* medium. It may be a simple salts solution with a carbon source, or it may include many organic components (Table 7-3).

NONSYNTHETIC. A medium in which the exact chemical composition is not known is called *nonsynthetic*. It is composed primarily of tissue extracts or infusions. An example is nutrient broth, which contains the following:

1. Peptone, a partially digested protein that serves

as a carbon and energy source as well as an important source of nitrogen
2. Beef extract, a source of vitamins and minerals
3. Sodium chloride, a salt that is not absolutely essential but does regulate the osmotic properties of the medium

Not many years ago the organic components just mentioned had to be obtained by the microbiologist, and the medium was prepared in one's own laboratory. Today most media or their individual components are commercially available. The preparation and function of various media can be found in suitable manuals of laboratory media (see Additional Readings). Because of the diverse chemical requirements of the microbial world, it is sometimes necessary to add or delete chemical components from prescribed laboratory media in order to isolate specific microbes. For this reason a classification of media based on specific laboratory use now exists.

Media Based on Laboratory Use

ALL-PURPOSE MEDIA. An all-purpose medium is designed to support the growth of most microorganisms. Examples of all-purpose media are nutrient broth, nutrient yeast, and trypticase soy broth. In the clinical laboratory trypticase soy broth is perhaps the most frequently used medium for maintenance of microbial populations.

ENRICHED MEDIA. Enriched media contain a basal growth-supporting medium to which are added nutritive supplements. Blood agar is an enriched medium widely used in clinical laboratories. Blood agar base contains an infusion of beef heart muscle, tryptose, salt, and agar. After sterilization this medium is allowed to cool to 50°C, and to it is added 5% sterile defibrinated sheep or horse blood. Fastidious microorganisms that will not grow on blood agar or other broths may require additional nutritive supplements to be cultivated.

SELECTIVE MEDIA. The purpose of a selective medium is to select for wanted species of bacteria and against unwanted species from a mixture. This is generally achieved in either a synthetic or a nonsynthetic medium by the addition or deletion of specific chemical components. For example, the removal of inorganic nitrogen ($[NH_4]_2SO_4$) from the prescribed basal salts medium described in Table 7-3 allows one to select for organisms capable of fixing gaseous nitrogen and against those that require nitrogen in the nongaseous state. Many other selective media incorporate dyes such as crystal violet or antibiotics to inhibit the growth of undesirable species. Other selective mechanisms that have been successfully employed are as follows:

1. Nutrient manipulation. The addition of specific carbon and energy sources can produce selective effects.
2. pH adjustments. While bacteria as a rule exhibit maximum growth in the pH range of 6.5 to 7.2, some groups of microorganisms prefer an acidic or basic medium. The fungi, for example, prefer a pH between 4 and 6.
3. Osmotic adjustments. The osmotic properties of a medium can be increased through the addition of nonmetabolizable compounds such as sodium chloride. The selection of halophilic (salt-loving) bacteria is enhanced by this procedure.
4. Oxygen tension adjustments. Increasing or decreasing the oxygen or carbon dioxide tension is important in the selection of anaerobes and aerobes. Many medically important pathogens have variable CO_2 requirements and cannot be cultivated unless the CO_2 tension is elevated above normal atmospheric levels.

DIFFERENTIAL MEDIA. A differential medium is designed to distinguish between various genera and species of microorganisms. Although two or more types of microorganisms can grow on this medium, some agent in the medium allows them to be differentiated. Often the separation is based on color differences in the isolated colonies. An example of this kind of medium is eosin—methylene blue (EMB) agar, which differentiates *Escherichia coli* from *Enterobacter aerogenes* primarily on the basis of color differences in their respective colonies.

Both of these organisms when grown on nutrient agar produce gray white colonies. On EMB agar, however, *E. coli* colonies are dark with a metallic sheen, while most *Enterobacter aerogenes* colonies are pink with a blue center and rarely have a metallic sheen. Most of the differential media are also selective and thus contain many kinds of growth-inhibiting agents.

ENRICHMENT MEDIA. In certain specimens (for example, fecal specimens) the infectious agent, such as species of *Salmonella*, may be greatly outnumbered by indigenous bacteria. An enrichment broth contains an agent that inhibits the unwanted species but favors the unrestricted growth of the infectious agent. Examples of enrichment media are tetrathionate and selenite broths.

TRANSPORT MEDIA. In some circumstances there may be a delay between the collection of a clinical specimen and its cultivation. Many microorganisms lose their viability when stored in ordinary laboratory media. Various transport media are available and consist of reducing agents such as thioglycolate or cysteine in a number of types of highly buffered salts media. An example of a transport medium is Stuart's medium. It is used in a dispensable culture unit called a *Culturette*, consisting of a sterile polystyrene swab and an ampule of the Stuart's medium. After the specimen has been collected, it is placed in the tube and the ampule is crushed to release the medium around the swab tip. This type of medium is not suitable for the isolation of oxygen-sensitive anaerobes.

FACTORS IN THE PREPARATION OF A CULTURE MEDIUM

The following factors are important in the preparation of a laboratory medium to be used for the isolation of microorganisms.

Fluidity

The dehydrated commercially available medium or the microbiologist's own concoction is first dissolved in water. If a solid medium is required, agar is then added.

pH

The pH of the medium is checked and adjusted if necessary. In commercial preparations adjustment is usually not necessary. In one's own synthetic media, phosphate buffers are generally used because the optimal growth of most bacteria is in the buffering range of the phosphate ions: 6.6 to 7.2.

Sterilization

The medium must be sterilized. This procedure ensures that all unwanted microorganisms present during the preparation of the medium are destroyed. If all components of the medium are stable to heat, sterilization is carried out in an autoclave (121°C, 15 pounds per square inch pressure for 15 minutes). If blood and certain unstable components such as antibiotics or heat-oxidizable compounds are required in the medium, they should be sterilized separately by filtration or other processes and then added to the sterilized medium when the latter has cooled to a temperature of 50°C.

Oxygen Tension

Successful isolation of microbial species depends on a knowledge not only of carbon and energy needs but of oxygen requirements as well. Attempting to isolate a strict anaerobe in the presence of oxygen would truly be an exercise in futility. Various methods have, therefore, been developed for the cultivation of bacteria based on their gaseous requirements.

ISOLATION OF AEROBES. Aerobic microorganisms, because respiration is their only source of energy, must have an adequate supply of oxygen in their environment. Oxygen demands can be satisfied in the following ways:

1. Agitating or swirling culture media in automatically controlled shaking devices can supply ade-

quate oxygen to the cells. Various shaking water baths provide agitation as well as temperature control for culture flasks. Aerobic organisms can also be cultivated without agitation. In the static procedure only those microorganisms at the surface will have oxygen available to them. Isolation of aerobes is most commonly accomplished, at least initially, in a diagnostic laboratory by simply inoculating the surface of a blood agar plate and incubating under atmospheric conditions.

2. Filtered air can be bubbled through culture vessels. Properly controlled, this method provides maximum distribution of oxygen throughout a culture medium. Some aerobic microorganisms require CO_2 tension for growth in culture. The increased demand for CO_2 (5 to 10% CO_2) can be met by a CO_2 generator (see Isolation of Anaerobes).

ISOLATION OF ANAEROBES. Many anaerobes, particularly nonsporeforming ones, are occasionally recognized as primary agents in infectious disease. These organisms are unusually delicate because of their sensitivity to oxygen. The cultivation and isolation of anaerobes play an important part in the diagnostic techniques used in the clinical laboratory. Their isolation requires proper specimen collection, specimen transport, and methods of cultivation under anaerobic conditions. Some of the chemical and physical techniques available to the microbiologist for the isolation of less sensitive anaerobes are as follows:

1. Reducing agents such as sodium thioglycolate, cysteine, or ascorbate can be added to the culture medium. Anaerobes can then be cultured in the presence of oxygen because the reducing agents absorb the free oxygen and make it unavailable to the bacteria.
2. Pyrogallic acid and sodium carbonate can be mixed in a small dish or flask, which is placed in a larger sealable container that holds culture flasks or agar plates. When the pyrogallic acid and sodium carbonate are mixed, oxygen is absorbed and CO_2 is released, thus creating an anaerobic environment. This technique is not used in the clinical laboratory.
3. The most widely used and least expensive way to isolate anaerobes is by the *anaerobic jar* method. The jars may be made of polycarbonate (GasPak), glass (Brewer), or steel (Torbal) and are equipped with a removable lid that can form an airtight seal. The GasPak system (Fig. 7-6) is ordinarily used in clinical laboratories. A specified amount of water is added to the contents of a disposable aluminum foil envelope. The envelope is placed in the GasPak jar along with the inoculated cultures. The jar is sealed, and the water added to the envelope generates the production of hydrogen and carbon dioxide gas.

Fig. 7-6. The GasPak for isolation of anaerobes. The GasPak is an anaerobic system consisting of a polycarbonate jar, lid holding a charged catalyst reaction chamber, GasPak disposable hydrogen-plus-carbon-dioxide generator envelope, and disposable anaerobic indicator. (Courtesy BBL Microbiology Systems, Division of Becton, Dickinson and Company, Cockeysville, MD.)

With the aid of a catalyst that is in the lid of the jar, any oxygen in the GasPak combines with hydrogen to form water, thus producing an anaerobic environment.

Other techniques such as the roll tube and anaerobic glove box are used for the isolation of the more sensitive anaerobes. They are discussed in Chapter 39.

Temperature

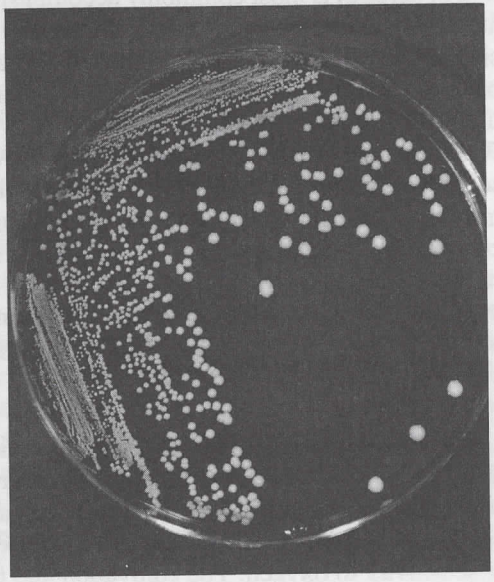
The medium must be incubated at a temperature that permits optimal growth of the bacterial species. Most clinical laboratories have incubators with automatic temperature controls. Knowing an organism's temperature requirements is just as important as any of the chemical and physical factors already discussed. Most pathogenic bacteria can be cultivated between the temperatures of 35° and 40°C with careful checks kept to safeguard against fluctuations. Some pathogens such as *Neisseria gonorrhoeae* are extremely temperature sensitive. Temperature as a factor affecting growth is discussed in Chapter 8.

LABORATORY ISOLATION

METHODS FOR ISOLATING BACTERIA

The identification of most microorganisms is dependent on a complete description of their morphological and biochemical characteristics. For identification procedures to be valid, it is important that only cultures containing one species of microorganism be used. Since in the majority of cases the organism to be isolated is part of a mixed population, the isolation of a single species from the environment is crucial. The isolation of a single species from a mixture of microorganisms is called the *pure culture* technique. The pure culture can also be referred to as an *axenic* culture. The following methods are used in the laboratory to obtain pure cultures. Each method can be enhanced by isolation on selective media if appropriate.

Fig. 7-7. The streak plate technique for isolation of colonies. The area of heavy growth indicates the initial site of inoculation. A second streak was made at right angles to the first, and a third streak at right angles to the second. As the growth is thinned out, individual cells are deposited on the agar, giving rise to discrete colonies.



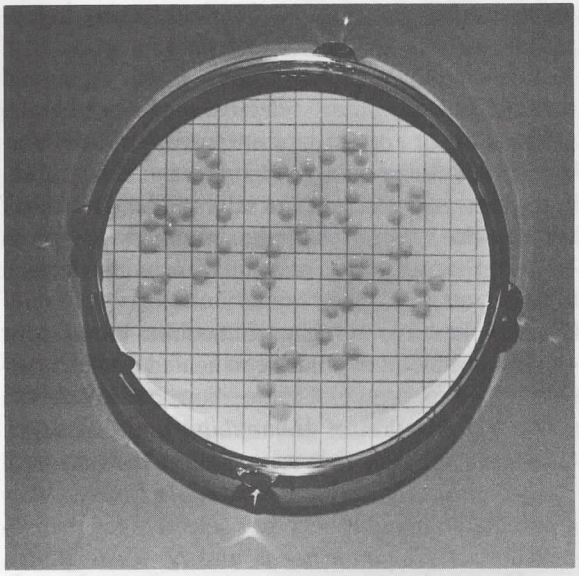
Streak Plate

In the streak plate technique a small quantity of the mixed population is streaked directly onto the surface of an agar medium. The streaking is performed with an inoculating loop. This procedure thins out the microbial population on the surface of the agar, and where an organism has been deposited, a colony will develop after suitable incubation (Fig. 7-7). From one such colony an inoculum is streaked onto the surface of a second agar plate, and the colonies that develop there are also examined. This subculture procedure ensures that only one kind of microorganism was present in the initially isolated colony.

Pour Plate

In the pour plate procedure the mixed population is first diluted and a small quantity is transferred to the bottom of a sterile Petri dish. Melted agar

Fig. 7-8. Colonies as they would appear on a membrane filter placed on an agar surface. (Courtesy Stanley Livingston.)



(50°C) is then poured into the Petri dish to cover the bacteria. The dish is gently tilted to obtain uniform distribution of organisms. Colonies will develop on the surface of the agar as well as in the agar. This technique may be used for the isolation of anaerobes or microaerophiles, but its usefulness is limited in the diagnostic laboratory. It is more commonly employed in the calculation of total numbers of organisms in milk and food.

Membrane Filter

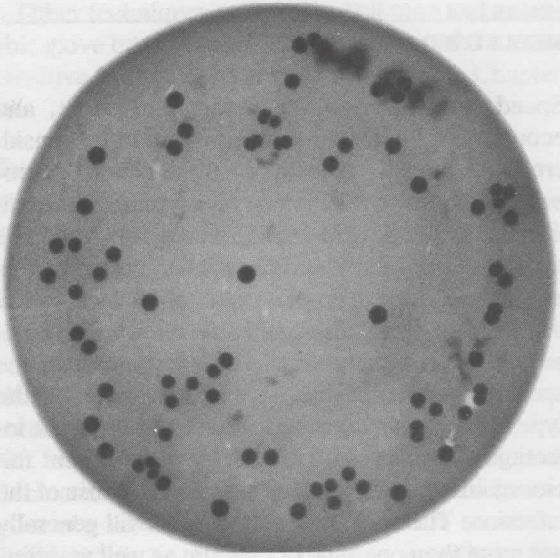
Membrane filters are thin porous sheets composed of cellulose esters or related materials. They have been used extensively in water and industrial microbiology in which large samples may contain few microorganisms. Once the sample is filtered, the filter can be placed on top of an agar medium, and after suitable incubation colonies will develop (Fig. 7-8). In clinical microbiology the membrane filter technique has some applications when (1) the organism may be in small numbers in a large volume of fluid, (2) the fluid from which the organism is being isolated is bacteriostatic (for example, spinal fluid or urine), and (3) total viable counts can be determined from urine samples and tedious dilution techniques avoided.

MEDIA USED FOR PRIMARY ISOLATION

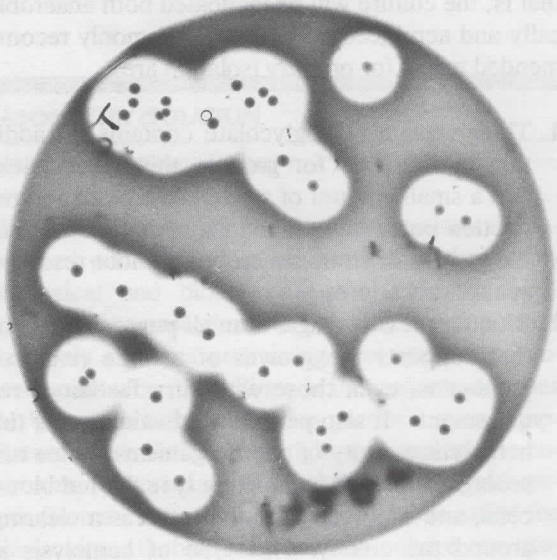
Speed, specificity of the testing procedure, and economy of the test are three factors to be considered in diagnostic procedures in the clinical microbiology laboratory. When an organism can be identified quickly and with certainty, the patient can be treated more rapidly and the probability of cure is enhanced. The choice of a medium for isolation of the microbial species is, therefore, extremely important. It is usually dictated by the source of the specimen—feces, urine, spinal fluid—and by the type of infectious agent suspected of causing the infection. A number of physiologically different microorganisms could conceivably be the cause of the infection. The clinical microbiologist will generally use more than one type of medium as well as different environmental conditions for primary isolation; that is, the culture will be incubated both anaerobically and aerobically. The most commonly recommended media for primary isolation are

1. Thioglycolate. Thioglycolate contains, in addition to nutrients for growth, thioglycolic acid and a small amount of agar. A low oxidation-reduction potential is thus maintained, permitting the growth of some anaerobes without resort to special procedures.
2. Blood agar. Blood agar is an all-purpose medium that supports the growth of a wide range of pathogens, even those with very fastidious requirements. It also permits the evaluation of the hemolytic activity of microorganisms. Some microorganisms will completely lyse the red blood cells, and on blood agar the result is a clearing around the colony. This type of hemolysis is called *beta hemolysis*. Incomplete hemolysis or *alpha hemolysis* causes a greening around the colony. Absence of hemolysis is called *gamma hemolysis* (Fig. 7-9).

Fig. 7-9. Hemolytic activity. A. Colonies show no hemolytic activity (gamma hemolysis). B. Colonies show beta-hemolytic activity. (From J. LeBlanc and L. N. Lee, *J. Bacteriol.* 150:835, 1982.)



A



B

3. Phenylethyl alcohol. Phenylethyl alcohol agar is a selective medium for the isolation of gram-positive cocci. The agents in this medium inhibit gram-negative species. Red blood cells may also be used in the medium for determining hemolytic activity of the coccal species.
4. Eosin–methylene blue agar. EMB agar and MacConkey agar are selective media used in the isolation of certain gram-negative species, particularly those that infect the intestinal tract. Agents in these media inhibit the growth of gram-positive species; the inhibitors are often dyes that are toxic and interfere with electron transport.
5. Chocolate blood agar. Chocolate blood agar contains blood that has been heated until the medium turns brown. This medium has been used for the isolation of *Hemophilus* and *Neisseria*. When antibiotics (vancomycin, colistin, and nystatin) are added to it, it is called Thayer-Martin medium and it becomes selective for the isolation of *N. gonorrhoeae* and *N. meningitidis*, the etiological agents of gonorrhea and meningitis, respectively.

PRESERVATION OF ISOLATED MICROORGANISMS

Many infectious microorganisms are extremely sensitive to environmental conditions outside the host. These species should be identified as quickly as possible. Most organisms can be preserved for future identification or experimental procedures. If, however, the original isolated colonies are left on the surface of the agar, they will become dehydrated and die within a few weeks. They must, therefore, be transferred to suitable media at regular intervals. A number of techniques can be used.

Subculture

The most traditional method of preservation is by subculture to growth-supporting media. Subculturing is done at regular intervals, which may vary from a day to several weeks depending on the species. After subculture to an agar surface (such as an

agar slant) or to a broth the specimen is stored at refrigeration temperatures (4°C). Even at 4°C the specimen is still metabolically active, and most of the cells will die within a few days or weeks. In addition, subculturing lends itself to possible contamination or loss of the culture via laboratory accidents.

Metabolic Reduction

The metabolism of microorganisms can be reduced by placing them in a medium containing minimal nutrients. Cultures are stabbed into such minimal agar media and then sealed with wax for preservation at refrigeration temperatures. An agar slant of the culture can be covered with liquid paraffin and stored for years. This technique has also been applied to the preservation of fungi. Sporeforming bacteria are often stored in sterile soil. The soil is first dried at room temperature and then stored at refrigeration temperatures.

Drying

Some bacteria can be preserved by drying. Heavy concentrations of the specimen are placed on wax disks or other suitable material and then transferred to a desiccator. The air in the desiccator is removed by a vacuum pump. After drying, the wax disks can be stored in sterile containers at 0–5°C.

Freezing

Most bacteria can be frozen in various types of media. The specimens are frozen rapidly to a temperature of −20° to −30°C to maintain the majority of cells in a viable state. Ultralow temperatures obtained by using liquid nitrogen have been successfully employed for storage and preservation of all biological cell types including fungi, viruses, and red blood cells.

Freeze-Drying (Lyophilization)

Ice crystal formation produced during the freezing process can damage cells. If the specimen is first frozen in a bath containing dry ice and acetone or some other mixture, the water in the cell can be removed by sublimation and damage to the cell prevented. This technique is commonly used for preservation of cultures and for shipment of cultures in the mail.

SUMMARY

Microorganisms have the same nutritional requirements as all living cells. These include carbon, nitrogen, sulfur, and phosphorous, which are needed for the organic building block material of the cell. The mineral requirement of microorganisms may vary from one species to another but usually includes magnesium, potassium, iron, calcium, zinc, molybdenum, cobalt, and manganese. Minerals are frequently cofactors for a number of enzymes in the cell. Some microorganisms cannot synthesize all of their organic needs, and they must be supplied in the medium. These organic needs are called growth factors and include amino acids, vitamins, and purine and pyrimidine bases. The vitamins are particularly important as coenzymes.

Microorganisms are divided into four basic groups based on the nature of their carbon and energy sources: photoautotrophs, photoheterotrophs, chemoautotrophs, and chemoheterotrophs.

To identify microorganisms obtained from clinical material they must be cultivated on appropriate media and isolated. Culture media may be of two kinds, agar or broth, and based on chemical content may be either synthetic or nonsynthetic. Most media used in the laboratory can be obtained commercially. Based on how the medium is to be used, it can be divided into different types, each having a specific purpose. These types include all-purpose media for isolation of most microorganisms, enriched media containing additional nutrients, selective media for isolation of wanted species, differential media for distinguishing between different species, enrichment media to facilitate growth of a wanted microorganism that is outnumbered by other microorganisms in the specimen, and transport media to maintain viability of microorganisms in a specimen that cannot be immediately transferred to other media.

Several factors must be taken into account during the preparation of laboratory media. The medium should be adjusted to the proper pH and must be sterilized to prevent contamination of the specimen. Once the medium has been prepared other factors must be controlled based on the physiological needs of the organism to be isolated. These factors include temperature and oxygen tension.

Isolation techniques in the laboratory are designed to obtain a single species from a mixture of microorganisms. This technique is called pure culture or axenic culture. The methods used to obtain a pure culture include streak plate, pour plate, and membrane filter. The choice of medium to be used in the primary isolation procedure is extremely important because you do not want to lose the microorganisms. Some important primary isolation media are thioglycolate, blood agar, phenylethyl alcohol, eosin–methylene blue (EMB) agar, and chocolate blood agar.

Once microorganisms have been isolated their identification may take some time; therefore, preservation of pure cultures is important. Preservation techniques include subculturing, metabolic reduction, drying, freezing, and freeze-drying.

QUESTIONS FOR STUDY

1. Why is it necessary that the cell use active transport for accumulating nutrients?
2. Distinguish between osmosis and active transport.
3. What nitrogen compound or compounds can be directly assimilated into organic material?
4. Name some important roles minerals play in microbial nutrition.
5. What is the function of vitamins in microbial nutrition?
6. How can microorganisms obtain energy from complex carbon compounds that cannot penetrate the cytoplasmic membrane?
7. Distinguish between a chemoautotroph and a chemoheterotroph in terms of energy and carbon needs.
8. What is the difference between an enriched medium and an enrichment medium? A selective medium and a differential medium?
9. What methods are available for obtaining a pure culture? What techniques would you use for a large urine sample?
10. What is the advantage of using thioglycolate broth as a primary isolation medium?
11. Under what circumstances is chocolate blood agar used?
12. What chemical reaction creates an anaerobic environment in the GasPak?
13. In what form is sulfur most frequently available to the bacterial cell in synthetic media? In what form is sulfur incorporated into the amino acids methionine and cysteine?
14. How does the bacterial cell store excess nitrogen?
15. How is the group translocation mechanism similar to active transport? Different from active transport?

ADDITIONAL READINGS

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8. MICROBIAL GROWTH AND FACTORS AFFECTING GROWTH

OBJECTIVES

To briefly describe how cell division is coordinated with DNA replication

To briefly describe the stages in the growth of a population of cells and the biochemical changes that occur

To explain what is meant by synchronous growth and how it can be obtained in the laboratory

To briefly describe the methods for measuring bacterial growth

To describe the physical factors affecting the growth of microorganisms

To define: binary fission, generation time, balanced growth, chemostat, spectrophotometer, psychrophile, mesophile, thermophile, lyophilization, plasmolysis

Growth in the Single Cell
Coordination of Cell Division with DNA Replication
Population Growth
Growth Rate
Growth Curve
Lag Phase
Log Phase
Stationary Phase
Death and Decline
Extension of Balanced Growth
Synchronous Growth
Laboratory Methods for Measurement of Growth
Cell Count
Cell Density
Cell Mass
Metabolic Activity
Physical Factors Affecting Growth
Temperature
Psychrophiles
Mesophiles
Thermophiles
Practical Aspects of Temperature Control
Hydrogen Ion Concentration
Radiation
Osmotic Pressure

We have already seen in Chapter 7 that there is a great diversity in the nutrient requirements for microorganisms. Nutrients are of obvious importance to the growth of the cell, but there are a variety of physical factors that also affect growth. An understanding of microbial growth is important because microorganisms affect our environment in a number of useful and nonuseful ways. As students of allied health professions, you should be concerned with microorganisms as agents of infectious disease. By controlling the growth of microorganisms we can control their infectious disease properties.

The purpose of this chapter is to examine how a single microbial cell as well as a population of cells grows and divides. Included in this discussion will be those physical factors affecting growth and how growth is measured in the laboratory. Chemicals used to control growth are discussed in Chapters 10 and 11.

GROWTH IN THE SINGLE CELL

The growth of a microbial cell is accompanied by an increase in cellular constituents and ultimately concludes with division of the cell into two equivalent cells (Fig. 8-1). Bacterial cell division is an asexual process referred to as *binary fission*. The division cycle in bacteria has been carefully analyzed by using various microscopical and genetic techniques. Much information concerning cell wall growth comes from studies on cocci that divide in one plane. Examination of a single coccal cell during division, as illustrated in Figure 8-2, has revealed the following:

1. The cell in the illustration is in the process of cell division, and a *cross wall* is being formed. But even before division of the cell two wall bands appear on either side of the cross wall. Mesosomes, which serve as sites of DNA attachment and division into nuclear areas, appear at these wall band sites. Cell wall synthesis begins at the new wall bands. Hydrolytic enzymes split the existing peptidoglycan, exposing new acceptor sites for addition of peptidoglycan precursors.
2. As the new cell wall is being synthesized it penetrates into the cytoplasm, producing a wall notch. As cell wall growth continues, the notch expands and the wall band splits apart into two layers of peripheral wall. Thus, a cross wall has been formed.
3. By the time the two layers of peripheral wall have been produced the initial cross wall has divided the cell into two cocci.

In rod-shaped bacteria there are apparently multi-

Fig. 8-1. Rod-shaped bacterium in the process of cell division. Arrow points to the site of cross wall (septum) formation. (From I. D. J. Burdett and R. G. E. Murray, *J. Bacteriol.* 119:1049, 1974.)

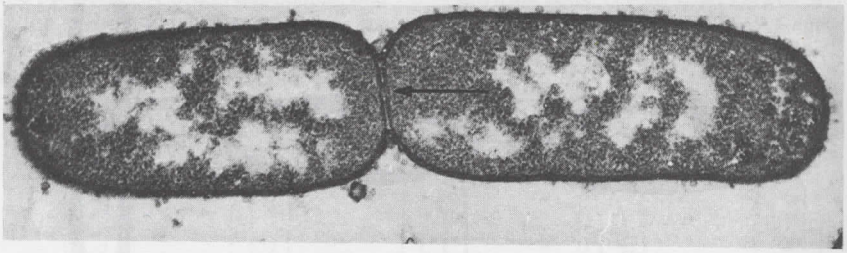


Fig. 8-2. Division of a coccal cell and its relationship to cell wall synthesis: cell division in *Streptococcus faecalis*. A. Cell is engaged in division in which a cross wall or septum has formed and nuclear material (*N*) has segregated. Note the attachment of the mesosome (*M*) to the nuclear material. B. As the division process continues, new sites of cell wall synthesis occur at wall bands (*circled areas*) (one for each future cell). Enlargement of the wall bands (*inset*) demonstrates where new cell wall synthesis begins. As the new cell wall is synthesized and expands, a wall notch, which represents the site of a new cross wall or septum, is produced. C. When the cell divides and daughter cells separate, each daughter cell also becomes engaged in cell division. (Adapted from M. L. Higgins and G. D. Shockman, *CRC Crit. Rev. Microbiol.* 1:29, 1971. Copyright © CRC Press, Inc., Boca Raton, FL.)

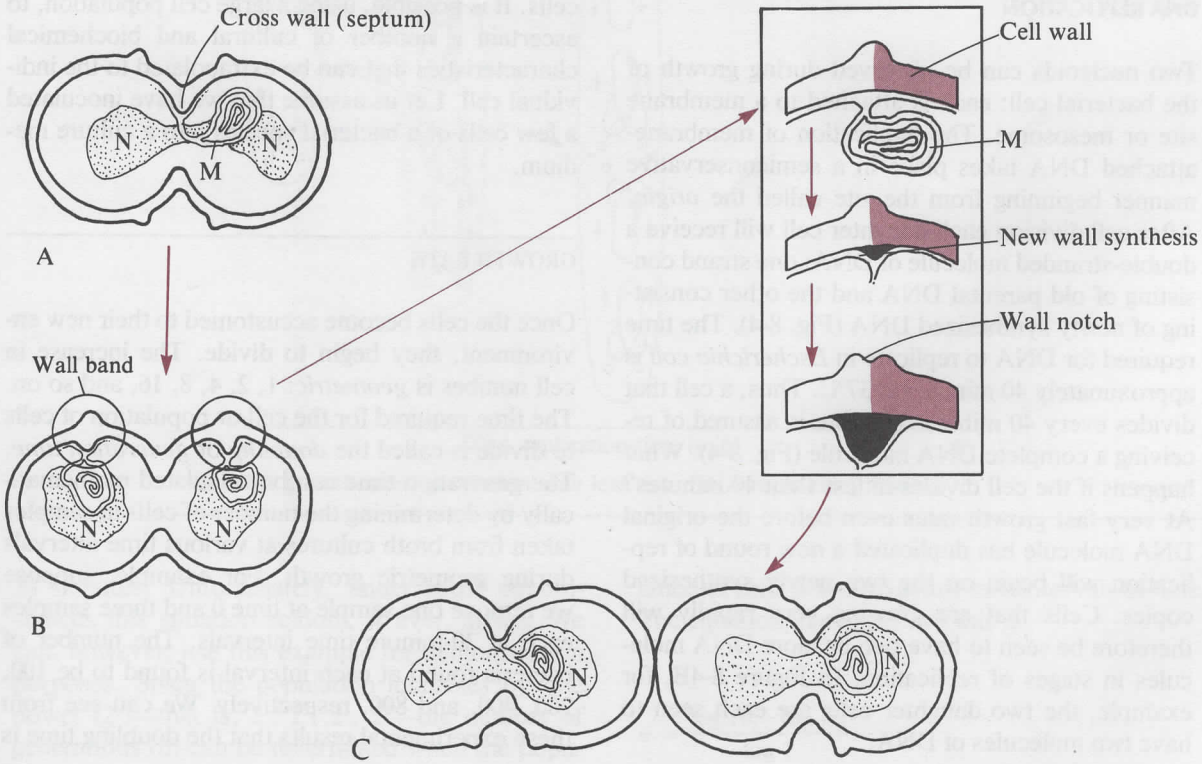
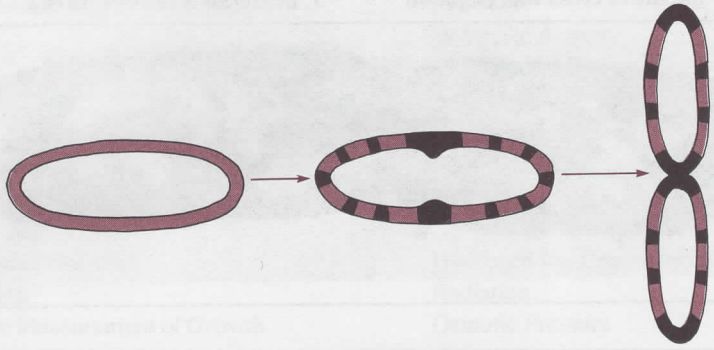


Fig. 8-3. Cell wall synthesis in a rod-shaped bacterium. The dark areas represent sites of new cell wall synthesis.



ple sites from which new cell wall is added (Fig. 8-3).

COORDINATION OF CELL DIVISION WITH DNA REPLICATION

Two nucleoids can be observed during growth of the bacterial cell; each is attached to a membrane site or mesosome. The duplication of membrane-attached DNA takes place in a semiconservative manner beginning from the site called the *origin*. After cell division each daughter cell will receive a double-stranded molecule of DNA: one strand consisting of old parental DNA and the other consisting of newly synthesized DNA (Fig. 8-4). The time required for DNA to replicate in *Escherichia coli* is approximately 40 minutes at 37°C. Thus, a cell that divides every 40 minutes or more is assured of receiving a complete DNA molecule (Fig. 8-4). What happens if the cell divides in less than 40 minutes? At very fast growth rates even before the original DNA molecule has duplicated a new round of replication will begin on the two newly synthesized copies. Cells that are growing very rapidly will therefore be seen to have two or more DNA molecules in stages of replication. In Figure 8-4B, for example, the two daughter cells are each seen to have two molecules of DNA.

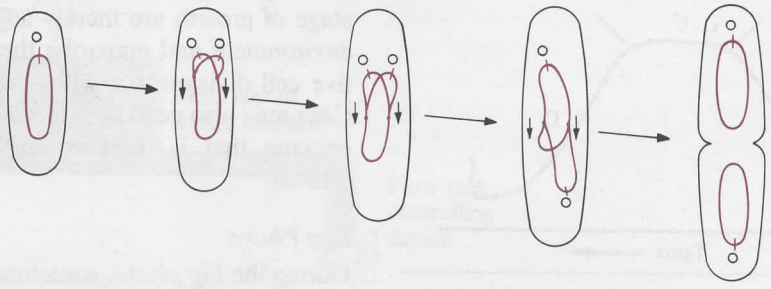
POPULATION GROWTH

The physical aspects of cell division can be measured microscopically using single cells, but studying the biochemical complexities of growth and cell division requires the use of large populations of cells. It is possible, using a large cell population, to ascertain a number of cultural and biochemical characteristics that can be extrapolated to the individual cell. Let us assume that we have inoculated a few cells of a bacterial species into a culture medium.

GROWTH RATE

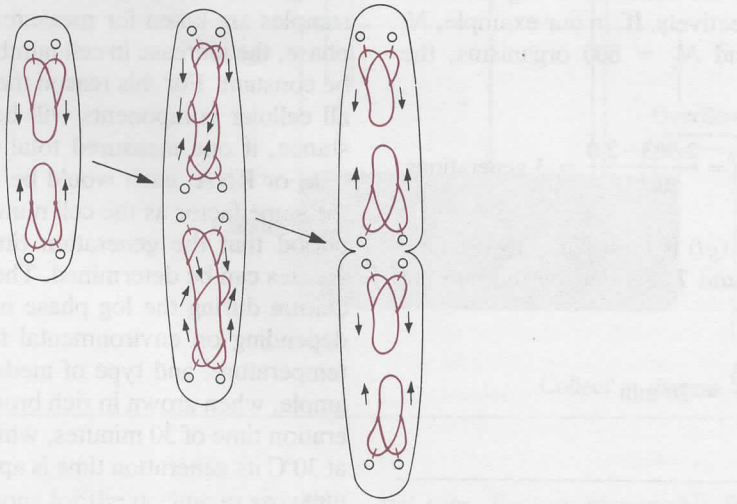
Once the cells become accustomed to their new environment, they begin to divide. The increase in cell number is *geometric*: 1, 2, 4, 8, 16, and so on. The time required for the cell or population of cells to divide is called the *doubling* or *generation time*. The generation time can be calculated mathematically by determining the number of cells in samples taken from broth cultures at various time intervals during geometric growth. For example, suppose we remove one sample at time 0 and three samples each at 30-minute time intervals. The number of bacteria found at each interval is found to be 100, 200, 400, and 800, respectively. We can see from these experimental results that the doubling time is

Fig. 8-4. Replication of bacterial DNA and its relationship to cell division. A. At low growth rates there is a single chromosome per cell. The origin duplicates, and there is no reinitiation at the origin before completion of a round of DNA synthesis. B. At high growth rates there are multiple initiations of replication at the origin before completion of a round of DNA synthesis. It is possible therefore to observe two or four nucleoids in rapidly growing cells. (From I. W. Dawes and I. W. Sutherland, *Microbial Physiology*. Oxford, England: Blackwell, 1976.)



○ is origin of replication

A. Low growth rates (generation time greater than DNA replication time)

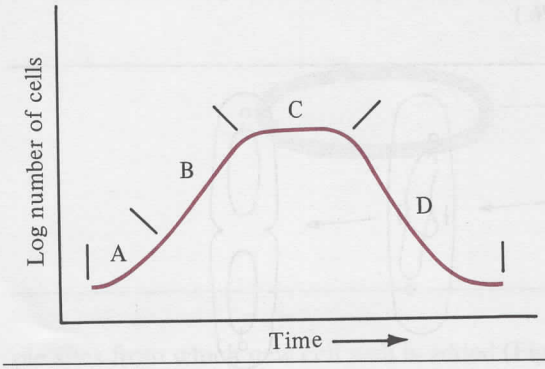


B. High growth rates (generation time less than DNA replication time)

30 minutes. Unfortunately, under most circumstances this situation seldom, if ever, arises. We can, however, use this example for demonstration purposes. Since the population increases by one power (2^n)—that is, 2^1, 2^2, 2^3, 2^4—the number of generations (n) can be determined when the population of cells is known at any two intervals of time. The equation that expresses this is

$$n = \frac{\log N_2 - \log N_1}{\log 2}$$

Fig. 8-5. Growth curve of a bacterial culture. A. Lag phase. B. Log or exponential phase. C. Stationary phase. D. Death-and-decline phase.



where N_1 and N_2 are the numbers of organisms at times T_1 and T_2, respectively. If, in our example, N_1 = 100 organisms and N_2 = 800 organisms, the equation reads

$$n = \frac{\log 800 - \log 100}{\log 2} = \frac{2.903 - 2.0}{0.301} = 3 \text{ generations}$$

The *generation time* (*gt*) is then equal to the time interval between T_1 and T_2 divided by the number of generations (n), or

$$gt = \frac{T_2 - T_1}{n} = \frac{90 \text{ min}}{3} = 30 \text{ min}$$

GROWTH CURVE

Suppose we were to remove samples of cells from a broth culture at regular intervals starting from the time of initial inoculation and continuing for several hours thereafter. If we determined the number of cells in the culture at each interval of time and plotted these data on semilog paper, a typical growth curve would be obtained (Fig. 8-5). The population of bacteria during the various stages of growth would exhibit definite morphological and biochemical characteristics.

Lag Phase

Cells taken (for instance) from an agar slant that has been stored at 4°C, or from any "old" culture, show an initial lag in cell division when placed in fresh broth medium. There is no lag, however, in the synthesis of macromolecules in the cell, particularly proteins and ribosomes. The cells at this stage of growth are merely adjusting to their new environment and preparing themselves for the active cell division that will occur in the log phase. Cells may also need time to synthesize an inducible enzyme that is required to initiate exponential growth.

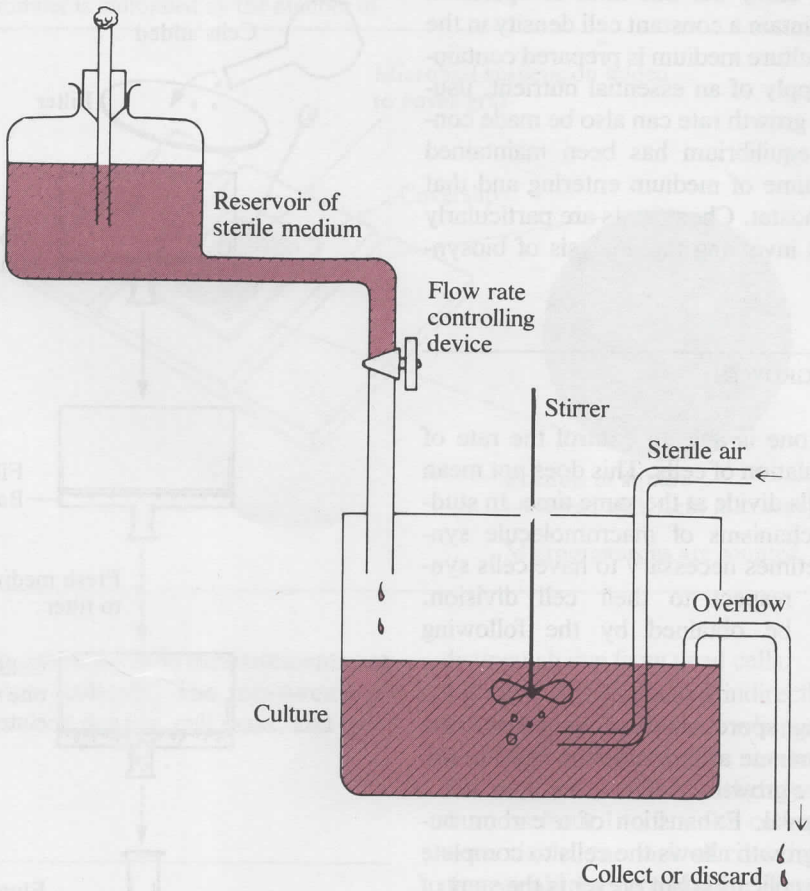
Log Phase

During the *log phase*, sometimes referred to as the *exponential phase of growth*, the cells are actively dividing at a uniform rate and are spoken of as being in *balanced growth*. No matter when two samples are taken for measurement during the log phase, the increase in cell number per unit time will be constant. For this reason the rate of increase for all cellular components will be the same. For instance, if one measured total cell mass, cell protein, or RNA, each would be seen to increase by the same factor as the cell number. It is during this period that the generation time of the microbial species can be determined. The rate of growth of a culture during the log phase is subject to change, depending on environmental factors such as pH, temperature, and type of medium. *E. coli*, for example, when grown in rich broth at 37°C has a generation time of 30 minutes, while in the same broth at 30°C its generation time is approximately 60 minutes.

Stationary Phase

It is certain that unless nutrients are eventually replenished in the log phase the rate of growth of the population will decline. When nutrients become depleted, cellular components no longer increase by the same factor, and the culture is spoken of as being in *unbalanced growth*. In this stationary phase there is a balance between the number of cells dying and the number dividing. This equalization is represented as a horizontal line in the growth

Fig. 8-6. Chemostat. (From J. Mandelstam and K. McQuillen, *Biochemistry of Bacterial Growth* [2nd ed.]. New York: Blackwell, 1973.)



curve (Fig. 8-5). Reasons for the decline in growth include (1) pH changes, (2) exhaustion of an essential nutrient, (3) accumulation of toxic waste products, and (4) reduced oxygen tension owing to overcrowding. It is during this phase of growth that some bacteria are capable of forming spores in response to the environmental change.

Death and Decline

The death-and-decline stage of growth is essentially the reverse of the exponential phase, that is, the cells in the population are dying at an exponential rate. Factors responsible for the death phase are the accumulation of toxic waste products, the release of various lytic enzymes, and loss of energy sources.

EXTENSION OF BALANCED GROWTH

We have seen that when nutrients become limiting in a medium the culture is no longer in balanced growth. If fresh medium is introduced at a constant rate and an equivalent amount of medium is re-

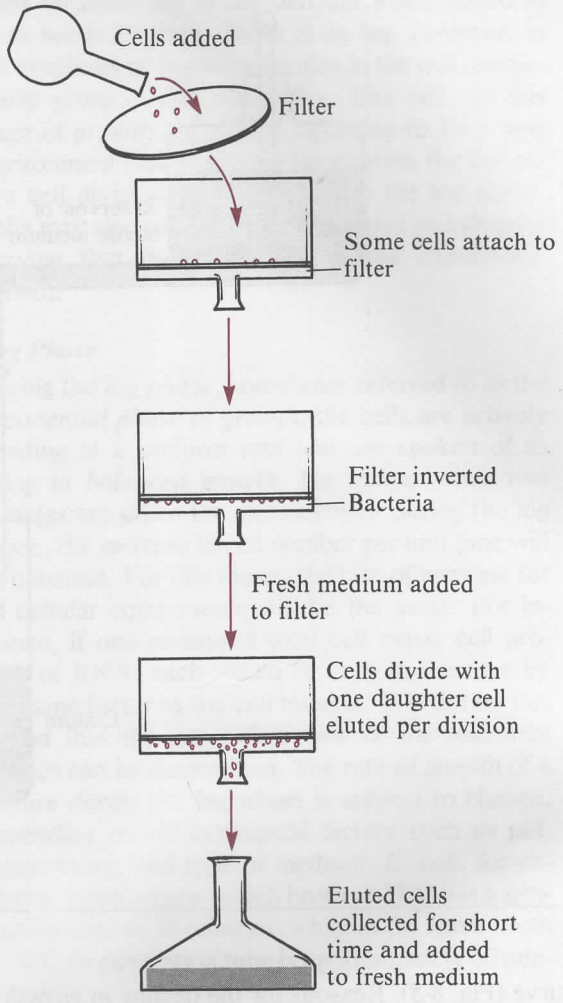
moved at a constant rate, balanced or logarithmic growth can be maintained indefinitely. Special apparatuses called *chemostats* or *bactogens* have been devised to carry out this kind of operation (Fig. 8-6). To maintain a constant cell density in the chemostat, the culture medium is prepared containing a limiting supply of an essential nutrient, usually carbon. The growth rate can also be made constant when an equilibrium has been maintained between the volume of medium entering and that leaving the chemostat. Chemostats are particularly useful in studies involving the analysis of biosynthetic pathways.

SYNCHRONOUS GROWTH

In a chemostat one is able to control the rate of growth of a population of cells. This does not mean that all of the cells divide at the same time. In studies probing mechanisms of macromolecule synthesis, it is sometimes necessary to have cells synchronized with respect to their cell division. Synchrony can be obtained by the following means:

1. Heat. Heating spore suspensions causes the spores to germinate at the same time and to initiate vegetative growth.
2. Nutrient removal. Exhaustion of a carbon nutrient during growth allows the cells to complete one round of replication but prevents the start of any new one. Reinitiation of growth by carbon addition results in synchrony.
3. Temperature control. Holding a culture at suboptimal temperatures for a period of time prevents rapid cell division. Elevation of the temperature can synchronize cells to divide at the same time.
4. Filtration. Certain filters permit the passage of small cells but not larger ones. Depending on the stage of growth, cells vary considerably in size. Before division the bacterial cell may be two to six times larger than cells produced just after division. The larger cells can be retained on the filter and when flushed with fresh medium will divide, giving rise to small cells that will pass through the filter (Fig. 8-7).

Fig. 8-7. Membrane filter technique for obtaining a synchronized culture.



LABORATORY METHODS FOR MEASUREMENT OF GROWTH

We have, up to now, discussed only the characteristics of bacterial growth. These characteristics can

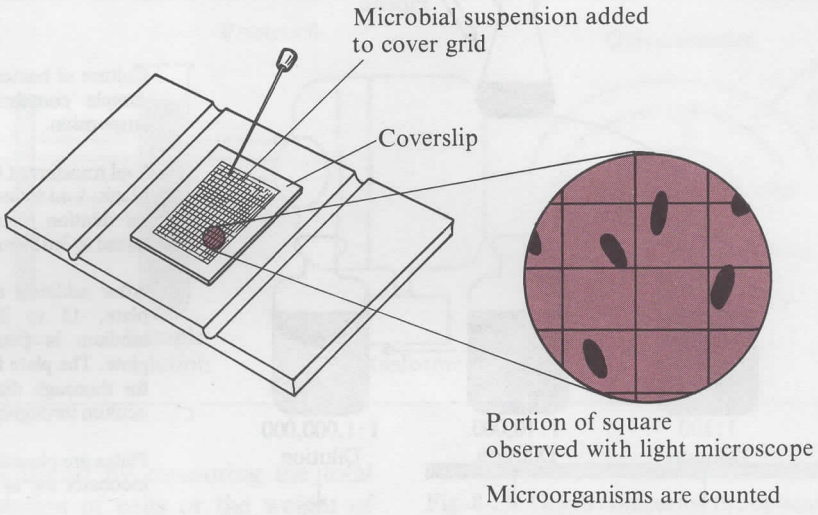


Fig. 8-8. Petroff-Hauser counter. The large square is observed microscopically after the addition of an aliquot of a culture. The volume of fluid on the large square is known, and this number is multiplied by the number of bacteria observed times the original dilution factor. The product is the concentration of bacteria per milliliter present in the culture sample.

be evaluated only when suitable measurements are made on laboratory cultures. The measurements include cell count, cell density, cell mass, and metabolic activity.

CELL COUNT

Using the microscope, one can count bacterial cells directly when a small portion of the culture is spread on a calibrated slide called the *Petroff-Hauser counter*. The Petroff-Hauser counter is similar to the hemocytometer used to count red blood cells. Calibrated ruled areas are etched into a special glass slide (Fig. 8-8). An aliquot of bacteria is introduced under the coverslip that covers the ruled area. The ruled area is observed microscopically, and the microorganisms are counted. Since the depth of fluid above the calibrated area is known, the number of organisms per unit volume can be calculated. This method cannot, however, distinguish live from dead cells.

Cells can be counted indirectly by determining the number of cells that, when spread on the surface of an agar plate, give rise to distinct colonies. The number of cells in a slightly turbid culture medium is about 1 to 2 $\times$ 10^7 cells per milliliter. If all were viable, the task of counting 10 million colonies would be formidable. The plate count technique usually involves some type of dilution that reduces the number of cells to a countable fraction (30 to 300 cells) before plating on a suitable agar surface. The colonies are counted, and that figure times the dilution factor will give the total number of bacteria per milliliter in the original culture before dilution (Fig. 8-9). This technique is one of the most widely used in microbiology laboratories. In addition to spreading directly onto the agar surface, the sample may also be filtered through a membrane filter that will retain the bacteria. The filter may then be placed over an agar surface and, after suitable incubation, the colonies counted (see Fig. 10-4).

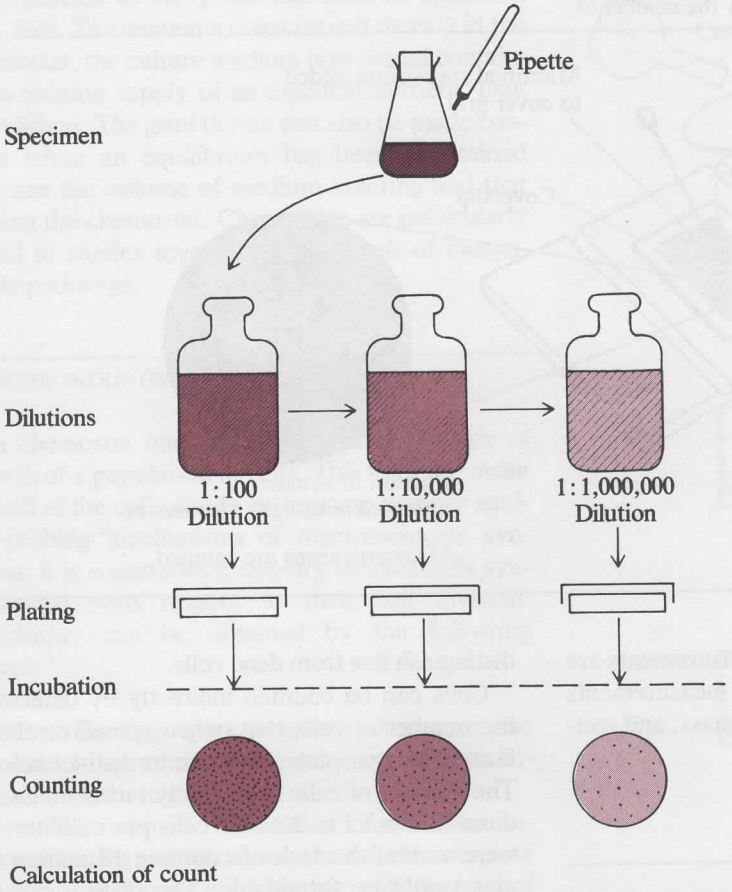


Fig. 8-9. Dilution technique for determining the viable cell number in a bacterial culture. (From M. J. Pelczar and R. D. Reid, *Microbiology*. 1972. Used with permission of McGraw-Hill Book Company.)

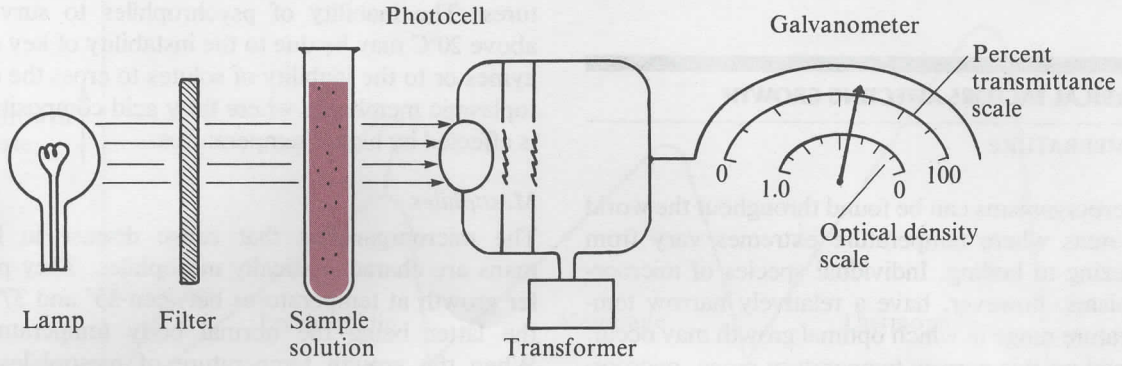
CELL DENSITY

As the population in a culture increases, the turbidity or optical density also increases. Changes in turbidity can be detected by a device called a *spectrophotometer*, which records them in units called *optical density* (*OD*) *units* or *absorbance* (*A*) *units* (Fig. 8-10). Other properties of the cell can be measured in conjunction with optical density measurements (Fig. 8-11). When the measurements are correlated and graphed, they can save the researcher a considerable amount of time. In subsequent experiments, for example, an optical density reading alone is required if the researcher wishes to know the population of cells. This technique can be applied only when one uses the same organism under the same environmental conditions and during exponential growth.

CELL MASS

One of the more quantitative determinations of cell growth is made by measurement of cell mass. Cell

Fig. 8-10. Spectrophotometer. A sample of bacteria in solution acts as a collodial suspension that intercepts light passing through it. Some light is absorbed; the remainder, passing through, activates a photocell, which in turn registers percent transmittance on a galvanometer. The higher the percent transmittance the fewer the cells in suspension. The amount of light absorbed by the suspension is reflected in the optical density. The greater the number of cells in suspension the greater the optical density.



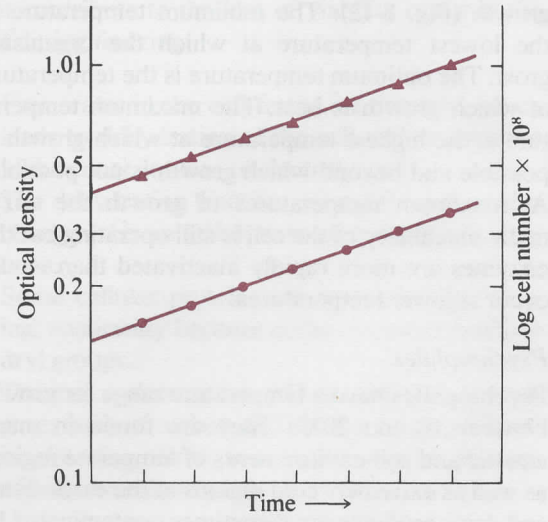
mass can be ascertained by measuring the total weight of a population of cells or the weight of some cellular component such as protein or RNA that shows a direct relationship to the cell mass. This is a rather time-consuming process and is used only in special research situations.

METABOLIC ACTIVITY

Sometimes in the clinical laboratory the rapid detection of microorganisms in urine is needed to determine the presence of infection. Some of these tests are based on the metabolic activity of the organisms, which is directly related to the number of bacteria in the specimen. These tests include measurement of bacterial adenosine triphosphate (ATP) by luciferase and production of radioactive CO_2 by metabolism of labeled substrates. In the former test, the firefly enzyme luciferase catalyzes the bioluminescent oxidation of $FMNH_2$ (reduced form of flavin mononucleotide) and a long-chain aldehyde by molecular oxygen:

$$FMNH_2 + O_2 + RCHO \xrightarrow[ATP]{\text{Luciferase}} FMN + H_2O + RCOOH + \text{light}$$

Fig. 8-11. Graph comparing the optical density (▲) and cell number (●) of a bacterial culture. Samples taken at indicated times were measured for optical density and cell number. Note that the rate of increase of both is the same.



This light-generating system competes for electrons with the normal cytochrome respiratory system. ATP is required for bioluminescence, and the light emitted, which is measured in a photometer, can be correlated with the ATP content of the cell.

PHYSICAL FACTORS AFFECTING GROWTH

TEMPERATURE

Microorganisms can be found throughout the world in areas where temperature extremes vary from freezing to boiling. Individual species of microorganisms, however, have a relatively narrow temperature range in which optimal growth may occur. Based on this narrow temperature range, microorganisms have been divided into three basic types: *psychrophiles, mesophiles,* and *thermophiles*. Psychrophiles are best suited to low temperatures, mesophiles to moderate temperatures, and thermophiles to high temperatures. When the growth rate of psychrophiles, mesophiles, and thermophiles is plotted, each group exhibits a characteristic minimum, optimum, and maximum temperature of growth (Fig. 8-12). The minimum temperature is the lowest temperature at which the organisms grow. The optimum temperature is the temperature at which growth is best. The maximum temperature is the highest temperature at which growth is possible and beyond which growth is not possible. At maximum temperatures of growth the enzymatic machinery of the cell is still operating but the enzymes are more rapidly inactivated than would occur at lower temperatures.

Psychrophiles

Psychrophiles have a temperature range for growth between 0° and 20°C. They are found in many aquatic and soil environments of temperate regions as well as extremely cold regions of the earth. Food and dairy products are sometimes contaminated by psychrophiles whose growth is stimulated when foods are refrigerated. Psychrophiles synthesize an increased proportion of unsaturated fatty acids when the temperature is lowered. This increase in unsaturation causes a decrease in the melting point of cytoplasmic membrane lipids. Thus fatty acids can be maintained in a more fluid state and normal cytoplasmic membrane activity, such as solute transport, can be maintained at the lower temperatures. The inability of psychrophiles to survive above 20°C may be due to the instability of key enzymes or to the inability of solutes to cross the cytoplasmic membrane where fatty acid composition is affected by higher temperatures.

Mesophiles

The microorganisms that cause disease in humans are characteristically mesophiles. They prefer growth at temperatures between 35° and 37°C, the latter being the normal body temperature. When the growth temperature of mesophiles is lowered, solute uptake is decreased. It is currently believed that low temperature produces conformational changes in the proteins of the cytoplasmic membrane, including transport proteins (permeases). Psychrophiles are more efficient in transporting solutes at the lower temperatures than are mesophiles.

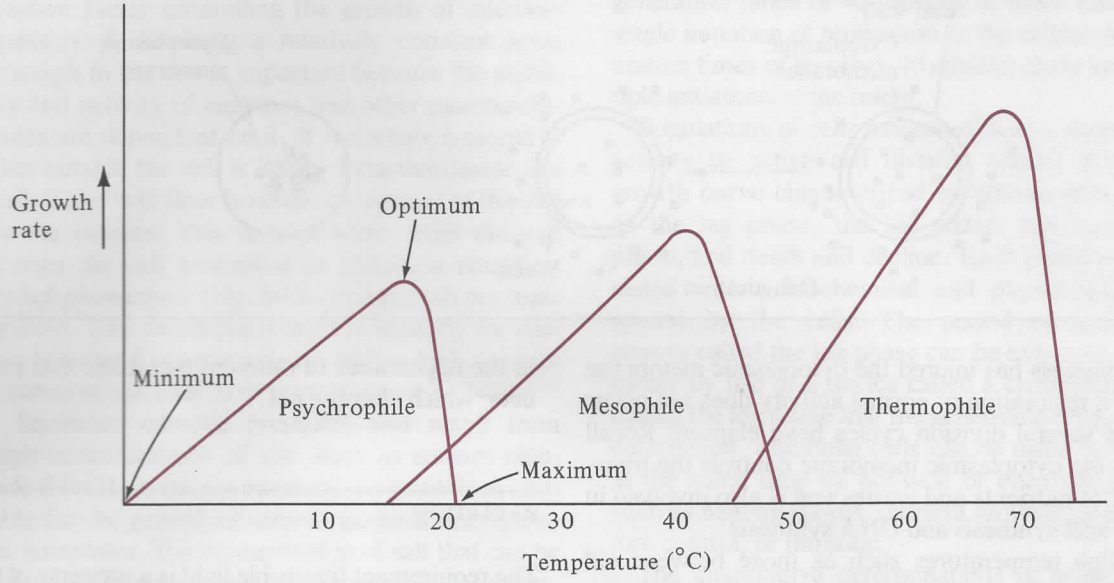
Thermophiles

Thermophiles show a temperature range for growth between 45° and 70°C. They are found in hot sulfur springs and other areas where high temperatures are maintained. Thermophiles are incapable of growth at the usual temperatures of the body and are not involved in infectious diseases of humans. The mechanism that enables thermophiles to resist elevated temperatures has not been resolved, but it is clear that their enzymes are more heat stable than the enzymes of mesophiles. This thermostability may be due to conformational changes in the enzymes, biochemical modification, or interaction of enzymes with cofactors. Resistance to high temperatures is a characteristic of spores, as was discussed in Chapter 4.

Practical Aspects of Temperature Control

Low temperatures are used as a technique for preservation of foods by inhibiting microbial growth.

Fig. 8-12. Comparison of the growth rates of psychrophiles, mesophiles, and thermophiles at minimum, optimum, and maximum temperatures.



Refrigeration temperatures (2–7°C) are suitable for the storage of a large number of perishable foods. Food-poisoning microorganisms, except for *Clostridium botulinum* type E, are prevented from growth at temperatures below 6°C. Still, many microorganisms can grow, albeit slowly, at temperatures from 0° to −34°C. Several yeasts, for example, are capable of growth at temperatures between −18° and −34°C. Generally speaking gram-positive cocci are more resistant to low temperatures than are gram-negative rod-shaped bacteria.

The freezing of microorganisms that cannot grow at freezing temperatures is a method for preserving microorganisms in the laboratory, as was mentioned in Chapter 7. Once freezing temperatures (−50° to −95°C) have been reached, a great percentage of the microorganisms die. Those cells surviving the initial freezing die gradually when stored in the frozen state. The rate at which they die depends on (1) whether the freezing is slow or fast, (2) the composition of the medium in which the microorganisms were frozen, (3) the length of storage in the frozen state, and (4) the temperature at which the microorganisms were frozen. Several changes that contribute to the loss of viability occur in the cell during freezing:

1. Free water in the cell forms ice crystals, which leads to dehydration of the cell and an increase in the concentration of electrolytes.
2. The dehydration from ice crystal formation also changes the colloidal state of cytoplasmic proteins.
3. Some cellular proteins are denatured by freezing, apparently because of the removal of sulfhydryl groups.
4. There is a decrease in pH as well as a loss of gases such as carbon dioxide and oxygen following freezing. Thus, metabolism and respiratory activity are suppressed.

Once the frozen culture has thawed many cells have increased nutritional requirements that remain for several generations. Apparently the freez-

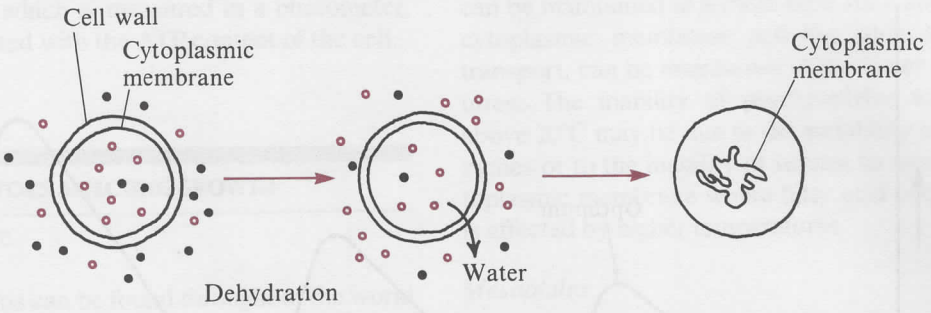


Fig. 8-13. Plasmolysis. Placing a bacterial cell in a hypertonic medium causes the removal of water from the cell. The dehydration causes the cell protoplast to shrink.

ing process has injured the cytoplasmic membrane and a restoration of normal activity does not occur until several division cycles have elapsed. Recall that the cytoplasmic membrane controls the transport of nutrients and wastes and is also involved in cell wall synthesis and DNA synthesis.

High temperatures such as those between 62° and 71°C are used in pasteurization processes, while temperatures above 100°C are used in sterilization procedures, which are discussed in Chapter 10.

HYDROGEN ION CONCENTRATION

The growth of microorganisms can be altered considerably by manipulating the hydrogen ion concentration (pH) of the environment. Bacteria collectively show a broad tolerance to the hydrogen ion concentration. Some favor a pH as low as 2.0, whereas others prefer a pH as high as 8.5. As a rule most bacteria exhibit optimal growth in the pH range of 6 to 8. Very low pH's are inhibitory to most infectious bacteria, and that is why the stomach represents a barrier to microbial colonization. Fungi, however, favor more acidic conditions and demonstrate optimal growth at pH's between 5 and 6. Although a bacterial species may grow at an alkaline as well as an acid pH, the products of metabolism may not be the same; in many cases the repression or induction of certain enzymes results in the appearance of different metabolic end products, which alter the pH.

RADIATION

The requirement for visible light is a property of the photosynthetic bacteria, algae, and plants. The ultraviolet portion of the electromagnetic spectrum penetrates the earth with sunlight. Some of the ultraviolet radiation is lethal to many microorganisms at higher altitudes above the earth's surface. Ultraviolet radiation has been used with great success in medicine and research, but these radiations are produced in various ultraviolet lamps. Shorter wavelengths in the ultraviolet range not only inhibit bacterial growth but can also be used to produce bacterial mutants that have proved invaluable as research tools in metabolic studies. Mutagenic effects are due primarily to the interaction of ultraviolet light with the cell's nucleic acids, especially DNA. X-rays and gamma rays are ionizing radiation and are lethal to most microorganisms because of the interaction of ions with large macromolecules. When ionizing radiation strikes the medium, ions and radicals such as the hydroxyl radical (·OH) are produced. The lethal effects of x-rays and gamma rays are due primarily to the interaction of these ions and radicals with DNA. See Chapter 10 for further discussion.

OSMOTIC PRESSURE

The concentration of solutes in a medium is an important factor controlling the growth of microorganisms. Maintaining a relatively constant ionic strength in the cell is important because the stability and activity of enzymes and other macromolecules are dependent on it. If the solute concentration outside the cell is higher than that inside the cell, water will flow from the cytoplasm of the cell to the outside. This loss of water from the cell causes the cell protoplast to shrink, a condition called *plasmolysis* (Fig. 8-13). Plasmolysis prevents growth. This technique is used in industry for curing hams and other meats by adding high concentrations of salt (NaCl) or sugar (sucrose).

Increased osmotic pressures that result from high concentrations of salt, such as sodium chloride (NaCl), in the environment are conditions suitable for the growth of microorganisms referred to as *halophiles*. The concentration of salt that can be tolerated is dependent on the microbial species. There are some microorganisms that actually require salt to survive. Members of the genus *Halobacterium* (a member of the archaebacteria) require high concentrations of salt to maintain the integrity of the cell wall. It is the sodium ion (Na^+) that stabilizes the cell wall of these bacteria, which lack a peptidoglycan normally associated with the true bacteria.

SUMMARY

The growth of a cell is associated with an increase in size, which is accompanied by cell division. The actual growth and division of the microbial cell has been examined with particular attention to cell wall synthesis and its coordination with DNA replication. In cocci a single wall band is produced from which new cell wall material is added peripherally, while in rod-shaped bacteria there are apparently multiple sites from which new cell wall is added.

Cell division and DNA replication are coordinated with the generation time of the cell. It takes approximately 40 minutes for DNA to replicate in *E. coli* at an incubation temperature of 37°C. At generation times of 40 minutes or more there is a single initiation of replication at the origin. At generation times of less than 40 minutes there are multiple initiations at the origin.

Populations of cells measured from a state of inactivity to active cell division exhibit a typical growth curve characterized by phases referred to as the lag phase, the log phase, the stationary phase, and death and decline. Each phase is associated with a biochemical and physiological response by the cells. The period of geometric growth called the log phase can be extended indefinitely by use of a device called a chemostat. It is during the log phase that the generation time can be determined. Microbial cells can be made to divide at the same time by physical or chemical means such as heating spores, nutrient removal, temperature control, or filtration.

The quantitative determinations of growth can be obtained by several direct or indirect techniques. These include measurements of cell count, cell density, cell mass, and metabolic activity.

The physical factors affecting growth include temperature, hydrogen ion concentration, radiation, and osmotic pressure. Microorganisms fall into three groups according to their optimum growth temperatures: psychrophiles (low temperature), mesophiles (moderate temperature), and thermophiles (high temperature). Bacteria have an optimum pH for growth between 6 and 8, while fungi are better adapted to more acid pH's. Ultraviolet light is damaging to microorganisms because it interacts with the DNA directly. Ionizing radiation produced by x-rays affects the DNA indirectly because x-rays generate ions when they strike the cytoplasm of the cell. These ions interact with DNA and other macromolecules. Most microorganisms remain viable when the solute concentration outside the cell is isotonic. Only a few microorganisms can exist in environments where the solute concentration outside the cell is hypertonic; they are called halophiles.

QUESTIONS FOR STUDY

1. What happens at the wall band during cell division in the cocci?
2. If the DNA molecule can replicate no faster than 40 minutes how does it keep up with cell division in a cell with a generation time of 20 minutes?
3. How does one determine the generation time of an organism growing in one type of medium at a specific temperature?
4. What is the difference between the stationary phase and the death-and-decline phase? The lag phase and log phase?
5. What is the purpose of a chemostat? How does it accomplish this task?
6. What cell count methods measure viable cells? Viable and dead cells?
7. What is the difference between optimum temperature and maximum temperature in terms of enzyme activity?
8. How does ultraviolet light affect the growth of a microbial cell?
9. What is meant by plasmolysis? Under what conditions does it occur?
10. What are halophiles, and where are they found?

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9. BIOSYNTHESIS AND METABOLIC CONTROL

OBJECTIVES

To briefly describe the source of precursors for the synthesis of amino acids, fatty acids, carbohydrates, and purines and pyrimidines

To explain how the glyoxylate cycle operates and its importance in metabolism

To explain what is meant by allosteric enzymes and their role in metabolism

To describe the ways in which feedback inhibition and energy-linked control affect metabolic pathways

To define: transamination, acyl-carrier protein, feedback inhibition, precursor, reducing power

Biosynthesis
- Biosynthesis and Genetic Potential
- Biosynthesis and the Source of Reducing Power
- Biosynthesis of Amino Acids
- Biosynthesis of Fatty Acids
- Biosynthesis of Carbohydrates
- Biosynthesis of Purines and Pyrimidines
- Biosynthesis During Growth on Two-Carbon Compounds: The Glyoxylate Cycle

Metabolic Control
- Regulation of Enzyme Activity
  - Allosteric Enzyme Kinetics
  - Feedback Inhibition
  - Energy-Linked Control

BIOSYNTHESIS

Biosynthesis is a metabolic process occurring in the cell that requires an expenditure of energy. The energy for biosynthesis is needed to produce precursor material that will be used in the formation of proteins, lipids, polysaccharides, and nucleic acids. The direct source of this energy, as we have already seen, may be light (in photosynthetic organisms) or inorganic or organic chemicals. Many cellular components and enzymes are rapidly turned over in the cell, and biosynthesis is needed to maintain them at their normal levels. The culmination of the cell's biosynthetic processes is cell division. Biosynthesis is also a process that is subject to various control mechanisms by the cell. Its rate is influenced by the availability of nutrients as well as various physical factors. The chemical and physical factors affecting growth were discussed in Chapter 8.

The purpose of this chapter is to outline the pathways used by the cell in the production of precursor materials: amino acids, carbohydrates, fatty acids, and organic bases that are used in the synthesis of specific polymers. In addition we will also discuss the mechanisms used by the cell to control the rate of biosynthesis.

BIOSYNTHESIS AND GENETIC POTENTIAL

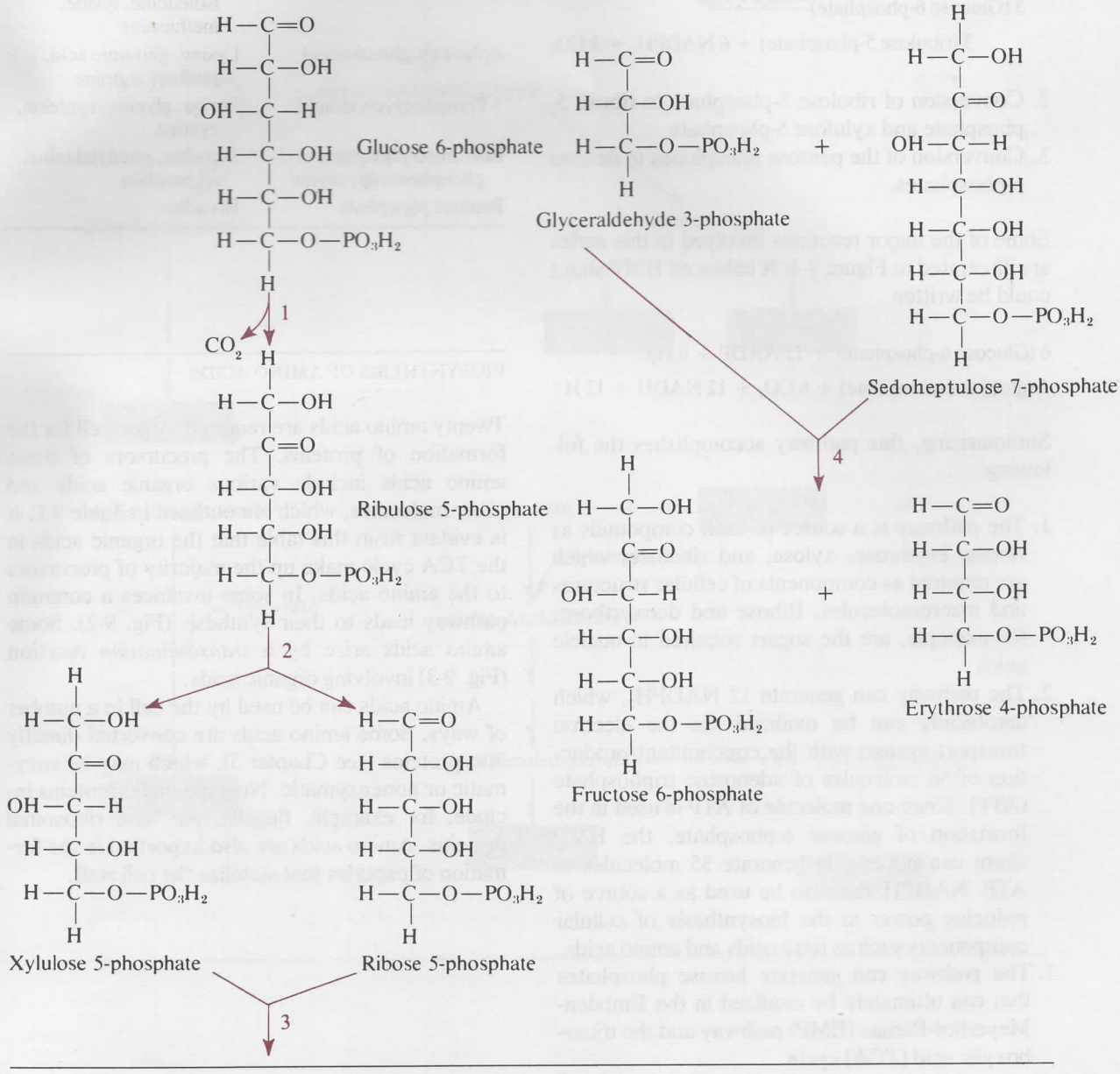
The ability of microorganisms to synthesize precursor material is related to their genetic potential. Some microorganisms such as *Escherichia coli* can grow in a minimal salts medium in which the carbon source is glucose. Glucose serves a dual role in the cell—energy can be extracted from it, and it can also be the carbon source. *E. coli* can, therefore, synthesize all of its precursor material from the glucose molecule. This is only possible because the *E. coli* chromosome codes for the enzymes that can carry out all of these biosynthetic processes. Many microorganisms are not genetically equipped to synthesize all their precursor material, and some precursors such as amino acids must be added to the growth medium (see Organic Growth Factors in Chapter 7).

BIOSYNTHESIS AND THE SOURCE OF REDUCING POWER

We have seen that the breakdown (catabolism) of chemical substances involves oxidation reactions that release energy. Biosynthesis, however, requires energy and entails reductions involving hydrogen. The source of this hydrogen or reducing power is primarily the reduced form of nicotinamide-adenine dinucleotide phosphate ($NADPH_2$), which is generated in a pathway called the *hexose monophosphate* (*HMP*) *shunt* or the *pentose phosphate cycle* (Fig. 9-1). This cycle, which is another avenue for the oxidation of glucose, is also the source of pentose phosphates for nucleic acid synthesis.

There are several reactions in the HMP shunt,

Fig. 9-1. Pentose phosphate pathway or hexose monophosphate shunt. Only the more important reactions are indicated in the scheme. In reaction 1, glucose 6-phosphate in a series of reactions is converted into ribulose 5-phosphate. In reaction 2, ribulose 5-phosphate may be converted to either xylulose 5-phosphate or ribose 5-phosphate, depending on the enzyme involved. In reaction 3, the two five-carbon units condense and are then rearranged into the three-carbon glyceraldehyde 3-phosphate and the seven-carbon sedoheptulose 7-phosphate. In reaction 4, the seven-carbon sugars and three-carbon sugars condense and then rearrange into the six-carbon fructose 6-phosphate and the four-carbon erythrose 4-phosphate.



but they can be grouped into three series of reactions:

1. Conversion of three molecules of glucose 6-phosphate in a series of reactions to ribulose 5-phosphate, a five-carbon sugar:

$$3\ (\text{Glucose 6-phosphate}) \xrightarrow{6\ NADP\ +\ 3\ H_2O} 3\ (\text{ribulose 5-phosphate}) + 6\ NADPH_2 + 3\ CO_2$$

2. Conversion of ribulose 5-phosphate to ribose 5-phosphate and xylulose 5-phosphate
3. Conversion of the pentose phosphates to hexose 6-phosphates

Some of the major reactions involved in this series are illustrated in Figure 9-1. A balanced HMP shunt could be written

$$6\ (\text{Glucose 6-phosphate}) + 12\ NADP + 6\ H_2O \rightarrow 5\ (\text{glucose 6-phosphate}) + 6\ CO_2 + 12\ NADH + 12\ H^+$$

Summarizing, this pathway accomplishes the following:

1. The pathway is a source of such compounds as ribose, erythrose, xylose, and ribulose, which are required as components of cellular structures and macromolecules. Ribose and deoxyribose, for example, are the sugars required in nucleic acids.
2. The pathway can generate 12 $NADPH_2$, which aerobically can be oxidized via the electron transport system with the concomitant production of 36 molecules of adenosine triphosphate (ATP). Since one molecule of ATP is used in the formation of glucose 6-phosphate, the HMP shunt can potentially generate 35 molecules of ATP. NADPH can also be used as a source of reducing power in the biosynthesis of cellular components such as fatty acids and amino acids.
3. The pathway can generate hexose phosphates that can ultimately be oxidized in the Embden-Meyerhof-Parnas (EMP) pathway and the tricarboxylic acid (TCA) cycle.

Table 9-1. Metabolic Intermediates Serving as Precursors in Biosynthesis of Amino Acids

Precursor(s)	Amino Acid End Product(s)
Pyruvic acid	Alanine, valine, leucine
Oxaloacetic acid	Aspartic acid, threonine, isoleucine, lysine, methionine
alpha-ketoglutaric acid	Lysine, glutamic acid, proline, arginine
3-Phosphoglyceric acid	Serine, glycine, cysteine, cystine
Erythrose-phosphate and phosphoenolpyruvate	Tyrosine, phenylalanine, tryptophan
Pentose phosphate	Histidine

BIOSYNTHESIS OF AMINO ACIDS

Twenty amino acids are required by the cell for the formation of proteins. The precursors of these amino acids include various organic acids and other molecules, which are outlined in Table 9-1. It is evident from this table that the organic acids in the TCA cycle make up the majority of precursors to the amino acids. In some instances a common pathway leads to their synthesis (Fig. 9-2). Some amino acids arise by a *transamination* reaction (Fig. 9-3) involving organic acids.

Amino acids can be used by the cell in a number of ways. Some amino acids are converted directly into proteins (see Chapter 5), which may be enzymatic or nonenzymatic. Nonenzymatic proteins include, for example, flagella, pili, and ribosomal proteins. Amino acids are also important in the formation of peptides that stabilize the cell wall.

Fig. 9-2. Pathways in which a variety of amino acids (*boxes*) are derived from oxaloacetate and pyruvate.

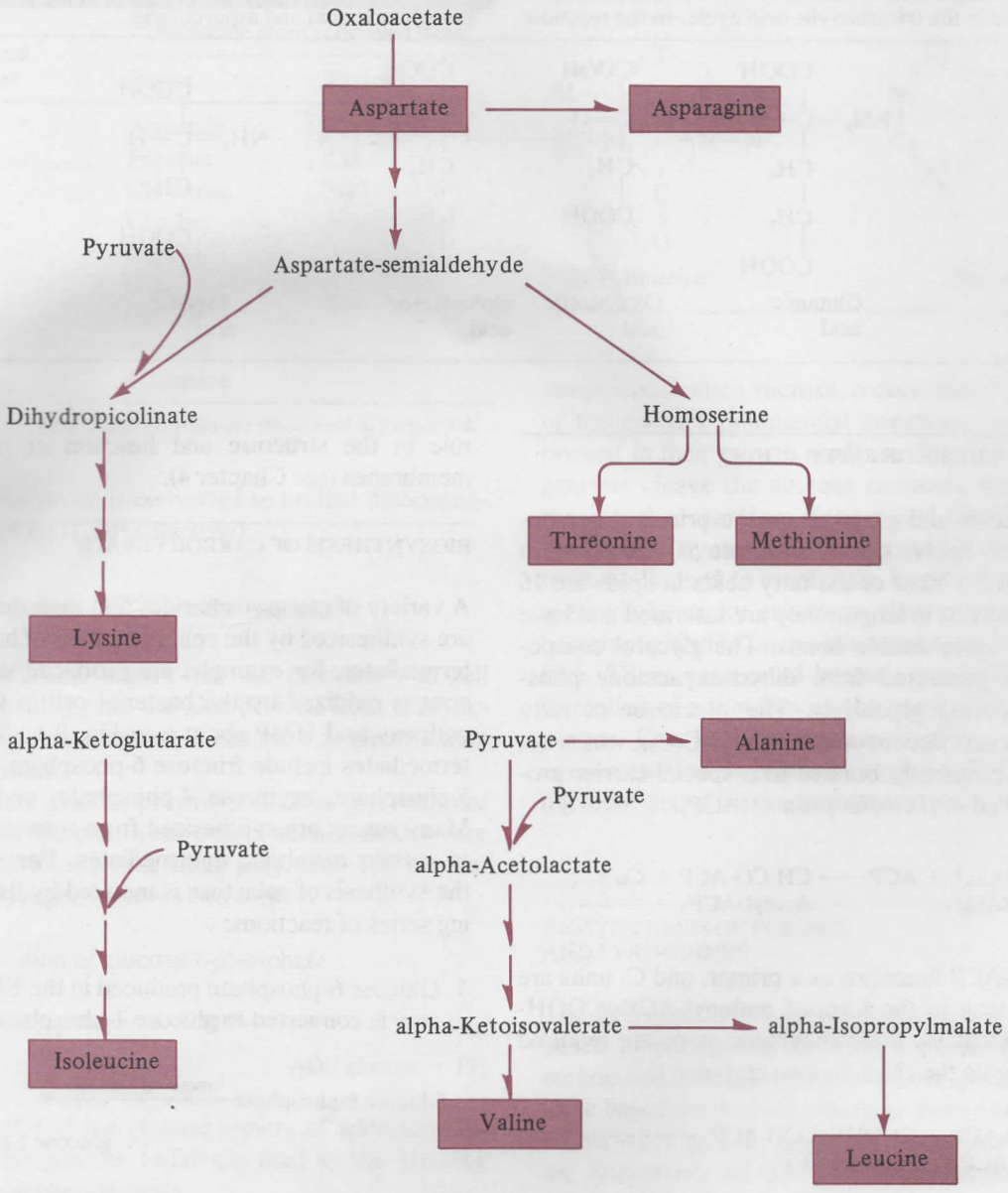
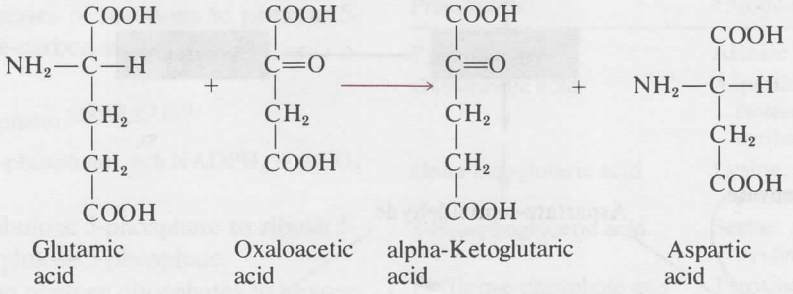


Fig. 9-3. Transamination, which is a cellular technique for producing different amino acids using keto-acids generated in the tricarboxylic acid cycle. In the reaction shown, the amino group of glutamic acid is transferred to oxaloacetic acid, resulting in the formation of alpha-ketoglutaric acid and aspartic acid.



BIOSYNTHESIS OF FATTY ACIDS

Fatty acids and glycerol are the principal components of lipids. (Lipid structure is discussed in Chapter 2.) Most of the fatty acids in lipids are 16 to 18 carbons in length; they are saturated and have one or more double bonds. The glycerol component is generated from dihydroxyacetone phosphate during glycolysis. The precursor of fatty acids is acetylcoenzyme A (acetyl-CoA), which becomes covalently bonded to a special carrier protein called *acyl carrier protein* (*ACP*):

$$\underset{\text{Acetyl-CoA}}{CH_3CO\text{-}CoA} + ACP \longrightarrow \underset{\text{Acetyl-ACP}}{CH_3CO\text{-}ACP} + CoA$$

Acetyl-ACP functions as a primer, and C_2 units are added to it in the form of malonyl-ACP ($COOHCH_2CO\text{-}ACP$). Four enzymatic steps are required to elongate the chain by two carbons:

$$\underset{\text{Acetyl-ACP}}{CH_3CO\text{-}ACP} + \underset{\text{Malonyl-ACP}}{COOHCH_2CO\text{-}ACP} \longrightarrow \underset{\text{Butyrl-ACP}}{CH_3CH_2CH_2CO\text{-}ACP} + NADP + CO_2$$

Elongation of the fatty acid chain then continues with two carbons added each time. Once the fatty acids are synthesized they are enzymatically linked to the glycerol molecule. Lipids play an important role in the structure and function of biological membranes (see Chapter 4).

BIOSYNTHESIS OF CARBOHYDRATE

A variety of monosaccharides and their derivatives are synthesized by the cell. A number of hexose intermediates, for example, are produced when glucose is oxidized by the bacterial cell in the EMP pathway and HMP shunt (see Fig. 9-1). These intermediates include fructose 6-phosphate, ribulose 5-phosphate, erythrose 4-phosphate, and others. Many sugars are synthesized from activated forms of certain metabolic intermediates. For example, the synthesis of galactose is initiated by the following series of reactions:

1. Glucose 6-phosphate produced in the EMP pathway is converted to glucose 1-phosphate.

$$\text{Glucose 6-phosphate} \xrightarrow{\text{phosphoglucomutase}} \text{glucose 1-phosphate}$$

2. Glucose 1-phosphate is activated by uridine triphosphate (UTP) to uridine diphosphoglucose (UDP-glucose).

$$\text{Glucose 1-phosphate} + UTP \xrightarrow{\text{UDP-glucose pyrophosphorylase}} \text{UDP-glucose} + \text{pyrophosphate}$$

Table 9-2. Origin of the Atoms of Purine and Pyrimidine Bases

Atom and position*	Molecule atom is derived from	
	Purine	Pyrimidine
N-1	Aspartate	Aspartate
C-2	Formate	CO_2
N-3	Glutamine	NH_3
C-4	Glycine	Aspartate
C-5	Glycine	Aspartate
C-6	CO_2	Aspartate
N-7	Glycine	—
C-8	Formate	—
N-9	Glutamine	—

*The atom number and position are illustrated in Figure 9-4.

3. UDP-glucose is converted to uridine diphosphogalactose (UDP-galactose).

$$\text{UDP-glucose} \xrightarrow{\text{epimerase}} \text{UDP-galactose}$$

Various amino sugars that are precursors to cell wall peptidoglycan are also derived from *UTP-activated* glucose intermediates such as glucosamine 6-phosphate.

Various glucose polymers such as glycogen are stored by the cell, while others such as dextran are synthesized as extracellular polymers. The synthesis of glycogen involves two steps:

1. Activation of glucose 1-phosphate

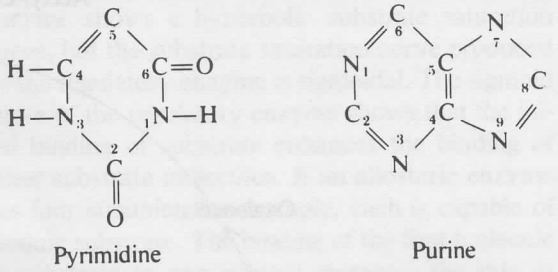
$$\text{Glucose 1-phosphate} + \text{ATP} \longrightarrow \text{ADP-glucose} + PP_i$$

2. Transfer of the glucose moiety of adenosine diphosphoglucose (ADP-glucose) to the growing polysaccharide chain

$$\text{ADP-glucose} + \text{glycogen}_n \longrightarrow \text{glycogen}_{(n+1)} + \text{ADP}$$

Dextran, a polymer of glucose, and levan, a polymer of fructose, are produced by certain oral streptococci when sucrose, a disaccharide and one of the primary commercial sweeteners (sugar), is present in their growth medium. The oral microorganisms cleave the sucrose molecule and release the monosaccharides, glucose and fructose. Glucose is used by the cell to synthesize dextran, while fructose is used to synthesize levan. Dextran is a particularly sticky substance that enables certain bacteria to bind to one another and to the tooth surface. These aggregated bacteria contribute to the formation of a thin film on the tooth surface called *plaque*. One bacterial species in plaque, *Streptococcus mutans*, is primarily responsible for initiating tooth decay (caries formation).

Fig. 9-4. Pyrimidine and purine, showing atom number and position.

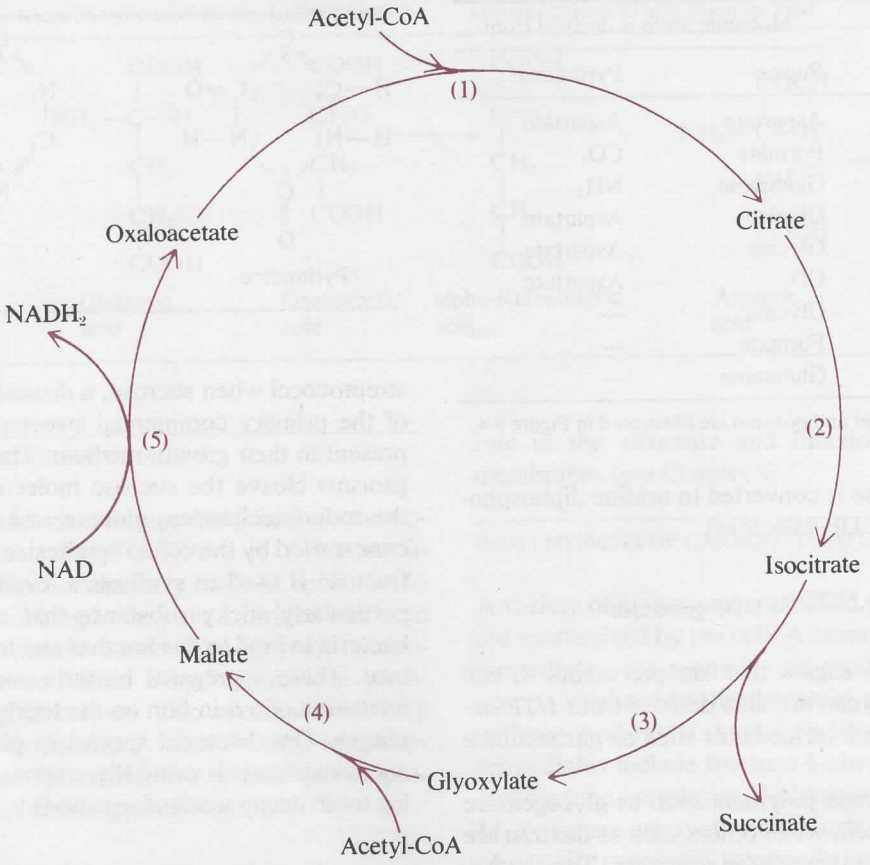


BIOSYNTHESIS OF PURINES AND PYRIMIDINES

The RNA and DNA polymers of the cell are composed of purine and pyrimidine nucleotides. The carbon and nitrogen atoms of the purine and pyrimidine bases are derived primarily from amino acids (Table 9-2; Fig. 9-4). Table 9-2 clearly demonstrates the importance of the TCA cycle–derived amino acids, especially aspartate. Many microorganisms can synthesize their own purine and pyrimidine nucleotides, but some cannot. Many oral bacteria, for example, require the addition of preformed purines or pyrimidines in order to grow on laboratory media.

The sugar phosphate component of nucleotides

Fig. 9-5. Glyoxylate cycle. See text for details.



is derived from intermediates in the HMP shunt discussed earlier.

BIOSYNTHESIS DURING GROWTH ON TWO-CARBON COMPOUNDS: THE GLYOXYLATE CYCLE

Some organisms are capable of growing on carbon sources more oxidized than glucose. Acetate, a two-carbon compound, is an example of a more oxidized carbon source. Recall that two carbons as CO_2 are released during one turn of the TCA cycle, and carbon would be unavailable for biosynthesis if unmodified acetate entered the cycle. Some bacterial species have evolved a mechanism whereby energy can be generated and carbons used for biosynthesis when they are growing on acetate as their only carbon source. This process is a bypass of the TCA cycle and is called the *glyoxylate cycle* (Fig. 9-5). The reactions in the cycle can be summarized as follows:

1. Two molecules of acetate are converted to acetyl-CoA, and one of the acetyl-CoA molecules condenses with oxaloacetate to produce a six-carbon molecule, citrate (reaction 1).
2. The six-carbon isocitrate molecule is cleaved to a four-carbon molecule, succinate, and the two-

carbon glyoxylate, with no loss of carbon (reaction 3).
3. A four-carbon unit, malate, is regenerated by condensing another molecule of acetyl-CoA with glyoxylate (reaction 4).
4. Potential energy is generated by the formation of $NADH_2$ (reaction 5).

METABOLIC CONTROL

For the cell to maintain an equilibrium between energy production and biosynthesis, control devices must be employed. What happens, for example, when the cell's ATP production is in excess of the amount it requires for biosynthesis? Will the cell continue to make more ATP and lose this energy as heat, or will it shut down energy production and wait for catabolic processes to fall in step with biosynthesis? Several different patterns of metabolic control exist, but basically they involve two types of regulation: regulation of enzyme synthesis, which was discussed in Chapter 5, and regulation of enzyme activity.

REGULATION OF ENZYME ACTIVITY

The metabolic requirements of a cell can fluctuate rapidly, and devices in the cell are available to control the activity of preformed enzymes. These control mechanisms are associated with enzymes that are composed of multiple subunits. Some of these subunits bind the enzyme's natural substrate at sites called *substrate-binding sites*; others bind metabolic products that control enzyme activity at sites called *regulatory sites*. These regulatory enzymes are also called *allosteric* enzymes. The regulatory sites on allosteric enzymes can bind small molecular weight molecules that increase or decrease the activity of the enzyme.

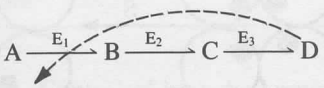
Allosteric Enzyme Kinetics

When the velocity of an enzymatic reaction is plotted against the substrate concentration, regulatory and nonregulatory enzymes show two types of enzyme kinetic curves (Fig. 9-6). The nonregulatory enzyme shows a hyperbolic substrate saturation curve, but the substrate saturation curve produced by the regulatory enzyme is sigmoidal. The sigmoid curve of the regulatory enzyme shows that the initial binding of substrate enhances the binding of other substrate molecules. If an allosteric enzyme has four subunits, for example, each is capable of binding substrate. The binding of the first molecule of substrate to one subunit increases the rate at which a second molecule of substrate binds to the second subunit, and so on, until the enzyme is saturated with substrate, that is, until all four subunits bind substrate (Fig. 9-7).

Allosteric enzymes are involved primarily in two types of cellular metabolic control mechanisms: (1) *feedback inhibition* or *end product inhibition* and (2) *energy-linked control.*

Feedback Inhibition

Feedback inhibition is the most common form of metabolic control in the cell and is associated primarily with biosynthetic reactions. This type of control assumes that when a product (or products) in a metabolic pathway begins to accumulate it can feed back on the first enzyme in the pathway and shut off not only the synthesis of the final product or products but also any intermediates in the pathway. In the following example, the final product D of the metabolic sequence feeds back and shuts off enzyme E_1.



In the absence of product B neither C nor D is produced. Enzyme E_1 in the sequence is therefore the allosteric enzyme.

Some metabolic pathways are branched, and more than one product is involved in the control mechanism. The cell must control the amounts of all the different products. Under some environmental conditions only one of the products may accumulate in the cell. Shutting down the first enzyme

Fig. 9-6. Comparison of the activities of regulatory and nonregulatory enzymes. At low substrate concentrations the curve is linear for nonregulatory enzymes but sigmoidal for regulatory enzymes. Only when the substrate concentration increases does a regulatory enzyme demonstrate nonregulatory kinetics.

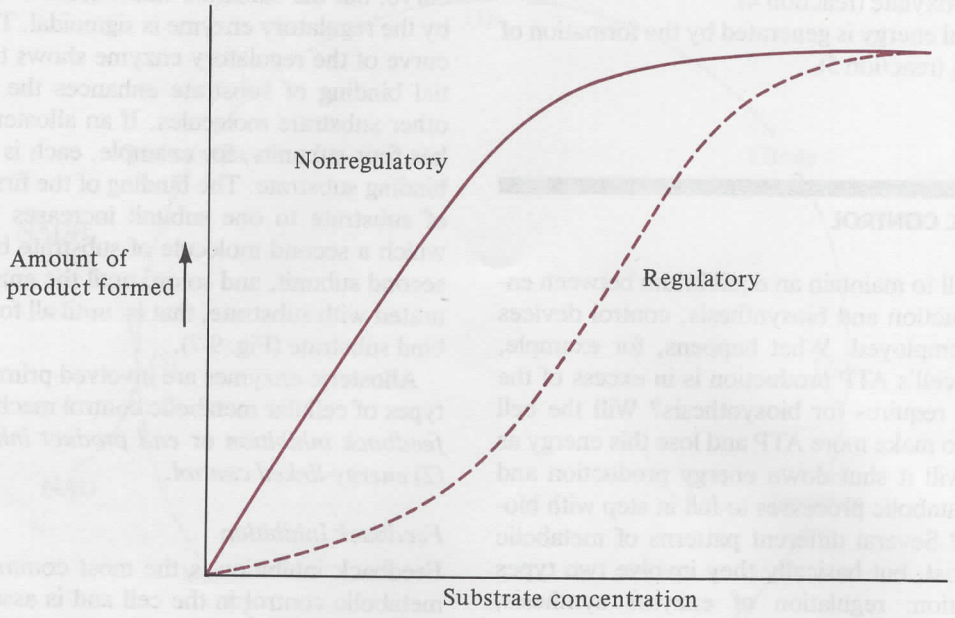
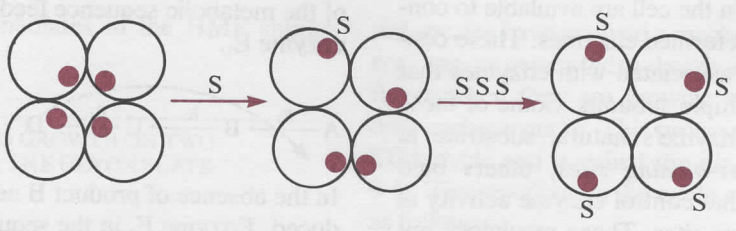


Fig. 9-7. Subunit interaction in allosteric enzymes. Substrate binding sites (●) on the subunits of the enzyme are hidden in the absence of substrate. Once a substrate molecule (*S*) binds to one subunit there is a conformational change in the enzyme molecule and the remaining binding sites become more accessible to substrate.



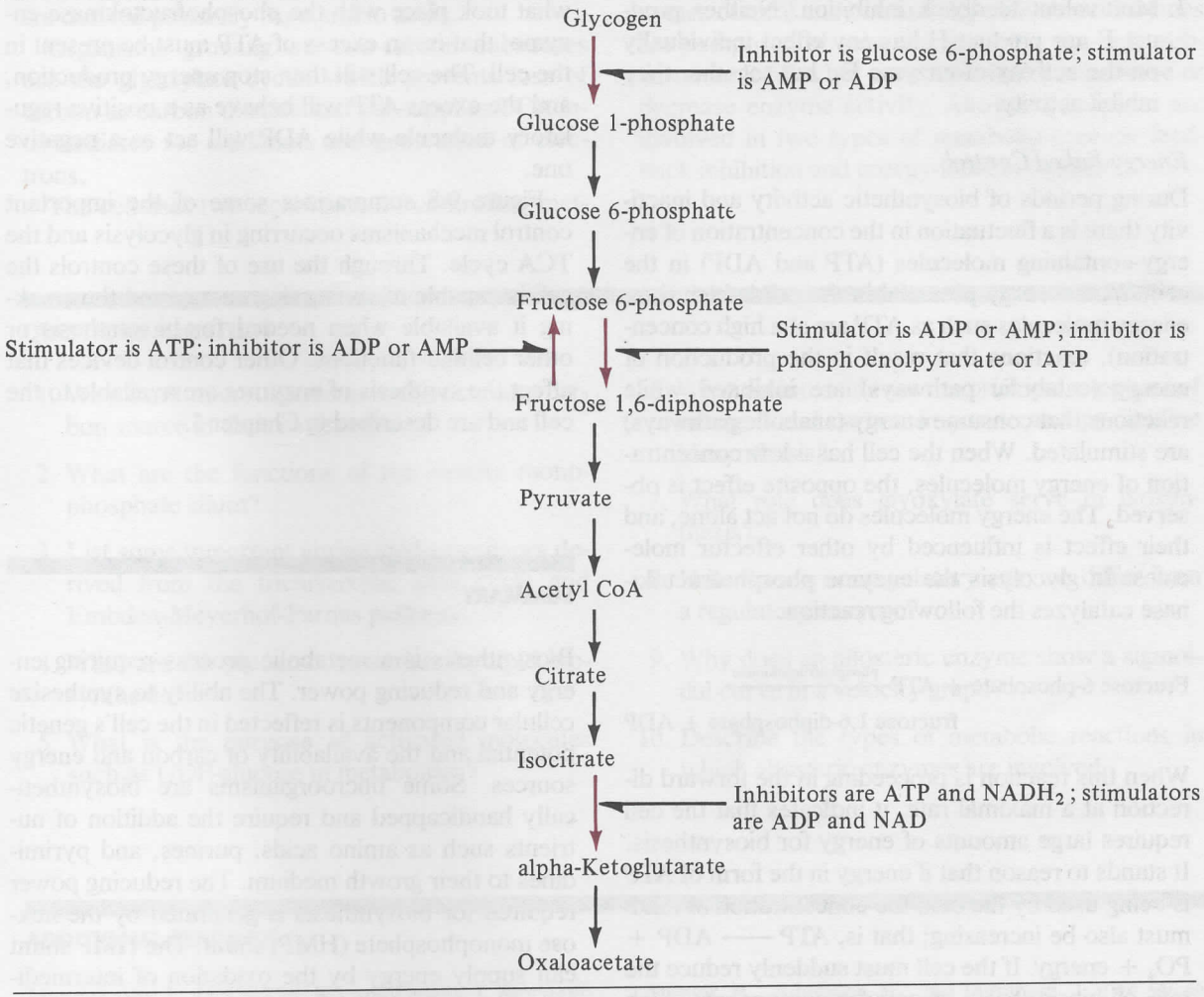
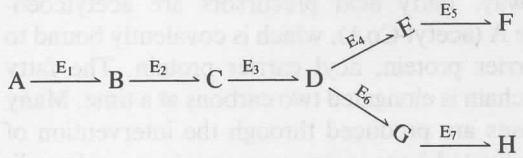


Fig. 9-8. Summary of the allosteric control sites in the glycolytic and tricarboxylic acid cycles. The regulatory molecules affect the reaction in the direction of the arrows. (AMP = adenosine monophosphate.)

will prevent the formation of both products. In the following example the primary products are D, F, and H, and the allosteric enzymes are E_1, E_4, and E_6:



Control of branched pathways can take place in the following ways:

1. Cumulative feedback inhibition. In the diagram neither product F nor product H alone could completely reduce the activity of the enzyme E_1; for example, each might reduce activity by 50 percent. Together, however, they totally shut off the activity of enzyme E_1.
2. Sequential feedback inhibition. Products F and

H feed back on enzymes E_4 and E_6, causing accumulation of product D. Product D in turn feeds back on enzyme E_1, inhibiting its activity.

3. Multivalent feedback inhibition. Neither product F nor product H has any effect individually on the activity of enzyme E_1, but together they inhibit activity.

Energy-linked Control

During periods of biosynthetic activity and inactivity there is a fluctuation in the concentration of energy-containing molecules (ATP and ADP) in the cell. When energy potential in the cell is high (i.e., energy molecules such as ATP are at a high concentration), reactions that result in the production of energy (catabolic pathways) are inhibited while reactions that consume energy (anabolic pathways) are stimulated. When the cell has a low concentration of energy molecules, the opposite effect is observed. The energy molecules do not act alone, and their effect is influenced by other effector molecules. In glycolysis the enzyme phosphofructokinase catalyzes the following reaction:

$$\text{Fructose 6-phosphate} + \text{ATP} \xrightarrow{\text{phosphofructokinase}} \text{fructose 1,6-diphosphate} + \text{ADP}$$

When this reaction is proceeding in the forward direction at a maximal rate, it indicates that the cell requires large amounts of energy for biosynthesis. It stands to reason that if energy in the form of ATP is being used by the cell, the concentration of ADP must also be increasing; that is, ATP $\longrightarrow$ ADP + PO_4 + energy. If the cell must suddenly reduce the rate of biosynthesis of cellular components, high concentrations of ATP will not be needed. Under these circumstances the ATP already produced will accumulate in the cell and ADP concentrations will decline. Excess ATP acts as a negative regulatory molecule and decreases the rate of the phosphofructokinase enzyme. In addition, phosphoenolpyruvate inhibits the activity of the enzyme (Fig. 9-8). The reversible reaction

$$\text{Fructose 1,6-diphosphate} + \text{ADP} \longrightarrow \text{fructose 6-phosphate} + \text{ATP}$$

is controlled by a separate enzyme called *fructose 1,6-diphosphatase*. If this reaction is to proceed at a maximal rate, the condition is just the opposite of what took place with the phosphofructokinase enzyme; that is, an excess of ATP must be present in the cell. The cell will then stop energy production, and the excess ATP will behave as a positive regulatory molecule while ADP will act as a negative one.

Figure 9-8 summarizes some of the important control mechanisms occurring in glycolysis and the TCA cycle. Through the use of these controls the cell is capable of conserving energy and thus making it available when needed for biosynthesis or other cellular functions. Other control devices that affect the synthesis of enzymes are available to the cell and are described in Chapter 5.

SUMMARY

Biosynthesis is a metabolic process requiring energy and reducing power. The ability to synthesize cellular components is reflected in the cell's genetic potential and the availability of carbon and energy sources. Some microorganisms are biosynthetically handicapped and require the addition of nutrients such as amino acids, purines, and pyrimidines to their growth medium. The reducing power required for biosynthesis is generated by the hexose monophosphate (HMP) shunt. The HMP shunt can supply energy by the oxidation of intermediates to pyruvate and is also important because it supplies pentoses needed for nucleic acid synthesis.

The principal precursors of amino acids are the organic acids produced in the tricarboxylic acid (TCA) cycle and the Embden-Meyerhof-Parnas pathway. Fatty acid precursors are acetylcoenzyme A (acetyl-CoA), which is covalently bound to a carrier protein, acyl carrier protein. The fatty acid chain is elongated two carbons at a time. Many hexoses are produced through the intervention of an activated hexose precursor such as uridine di-

phosphoglucose (UDP-glucose). Pentoses are produced mainly in the HMP shunt. The carbon and nitrogen components of purines and pyrimidines are derived primarily from amino acids.

Organisms growing on two-carbon substrates use the glyoxylate cycle, which prevents loss of carbon as carbon dioxide and yet supplies TCA intermediates for oxidation and generation of electrons.

The cell uses two techniques for controlling metabolic reactions: regulation of enzyme synthesis (discussed in Chapter 5) and regulation of enzyme activity. Regulatory enzymes called allosteric enzymes control enzyme activity. Allosteric enzymes are composed of subunits that bind not only substrate molecules but molecules that can increase or decrease enzyme activity. Allosteric enzymes are involved in two types of metabolic control: feedback inhibition and energy-linked control.

QUESTIONS FOR STUDY

1. Why can't microorganisms catabolize any carbon source in their environment?
2. What are the functions of the hexose monophosphate shunt?
3. List some important amino acid precursors derived from the tricarboxylic acid cycle and Embden-Meyerhof-Parnas pathway.
4. What are the precursor materials for lipid biosynthesis?
5. What is the purpose of activated molecules such as UDP-glucose in metabolism?
6. What amino acids are the principal suppliers of nitrogen and carbon in purine and pyrimidine biosynthesis?
7. What role does glyoxylate serve in biosynthesis?
8. How does a nonregulatory enzyme differ from a regulatory enzyme?
9. Why does an allosteric enzyme show a sigmoidal curve in a velocity graph?
10. Describe the types of metabolic reactions in which allosteric enzymes are involved.

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II. CONTROL OF MICROORGANISMS

10. STERILIZATION AND DISINFECTION

OBJECTIVES

To define the commonly used sterilization/disinfection terms and use them in specific contexts, and especially to differentiate between antisepsis and disinfection

To appraise conditions or factors that affect sterilization/disinfection procedures and agents

To name the commonly used modes of attaining sterility; to point out the advantages and the limitations of each; for each mode to state the minimum conditions that must be applied to assure the attainment of sterility

To give the mechanisms of action, the good and bad features, and the typical applications of moist and dry heat, cold, filtration, ultrasound, and radiation as they pertain to the control of microorganisms

To comment on sterilization indicators as to their composition, rationale for use, application, interpretation of readouts, and reliability

To give the desirable properties of disinfectants and describe their mechanisms of action

To give the mechanisms of action, the good and bad features, and the typical applications of representative disinfectants from each of the major chemical disinfectant groups; specifically include hexachlorophene, 70 percent alcohol, bleach, tincture of iodine, iodophors, cationic detergents, ethylene oxide, glutaraldehyde, and chlorhexidene

To cite some of the problems associated with disinfectant evaluation and describe and critique the phenol coefficient test

- Terminology
  - Sterility
  - –cide
  - –stasis
  - Sepsis
  - Asepsis
  - Antisepsis
  - Disinfection
  - Sanitization
  - Degermation
  - Chemotherapeutic Agents
- General Considerations
  - Population Unknown
  - Determination of Level of Kill
  - Variations Within a Population
  - Problem Microorganisms
    - Bacterial Endospores
    - Mycobacteria
    - Hepatitis Viruses
    - *Pseudomonas*
  - Factors That Affect the Activity of Antimicrobial Agents and Procedures
    - Interfering Matter
    - pH
    - Other Environmental Factors
  - No Universal Method
  - Careless Handling
  - Effects on Bacteria as the Principal Criteria
  - Principal Modes of Achieving Sterility
- Physical methods
  - Heat
    - Comparison of Heat Tolerance Levels of Microorganisms
      - Thermal Death Point
      - Thermal Death Time
      - Rate of Death Determinations
    - Moist Heat
      - Boiling
      - Steam at Atmospheric Pressure
      - Steam Under Pressure—Autoclaving
        - General Advisements About Autoclaving
        - Sterilization Indicators
      - Pasteurization
    - Dry Heat
      - Dry Heat Sterilization
      - Incineration
  - Cold
    - Refrigeration
  - Filtration
    - Bacteriological Depth Filters
    - Membrane Filters
    - High-Efficiency Particulate Air Filters
  - Ultrasonics
    - Ultrasonic Cleaners
    - Mechanism of Action
    - Other Ultrasound Applications
  - Radiation
    - Ultraviolet Radiation
      - Irradiation of Room Air
      - Limitations of Ultraviolet Radiation
    - Ionizing Radiation
      - Advantages and Disadvantages
      - Applications
- Chemical Methods
  - Qualities of a Universal Disinfectant
  - Mode of Action
    - Cell Membrane Injury
    - Coagulation and Denaturation
    - Interactions with Functional Groups of Proteins
    - Oxidation
  - Design of Disinfectants
  - Evaluation of Disinfectants
    - Initial Testing
    - In-Use Testing
    - The Phenol Coefficient Test
      - Phenol Coefficient Determinations
  - Classes of Disinfectants
    - Phenol and Phenolics
      - Hexachlorophene
      - Other Phenolics
    - Alcohols
    - Halogens
      - Chlorine
      - Iodine
        - Tinctures of Iodine
        - Iodophors
    - Surfactants
      - Soaps

Detergents
Anionic Detergents
Cationic Detergents
Nonionic Detergents
Alkylating Agents
Formaldehyde
Glutaraldehyde
Ethylene Oxide
Betapropiolactone
Heavy Metals
Other Classes of Disinfectants
Selection of Disinfectants
Level of Disinfection
Trade or Proprietary Name
Practical Recommendations

Infectious disease agents are controlled in four ways: by public sanitation measures, by sterilization and disinfection procedures, by chemotherapeutic agents, and by the body's defensive mechanisms. Public sanitation methods are used to control the sources of microorganisms by such measures as proper disposal of wastes and purification of water. Sterilization and disinfection procedures seek to destroy microorganisms before they have an opportunity to enter the body. Chemotherapeutic measures (see Chapter 11) are used to affect microorganisms once they have entered the body. The body's nonspecific resistance mechanisms and its immune system (see Chapters 13 to 16) either prevent microorganisms from entering or, once they have entered, remove, inactivate, or destroy them.

TERMINOLOGY

The terms *sterilization* and *disinfection* have precise meanings but in casual conversation are loosely used. Some imprecision arises from the fact that certain words (particularly *sterilize* and *disinfect*), although they have a definite meaning in a restricted sense, also serve as general terms for other antimicrobial procedures and agents. The word *disinfectant*, for example, in many contexts represents all the chemical agents that are employed for disinfection, antisepsis, sanitization, and degermation. *Sterilize* often is used in a general way to signify any antimicrobial treatment, even though the objective of the treatment is not necessarily to achieve sterility. In place of *antisepticize*, or *disinfect* one frequently hears *sterilize* when the type of treatment used or requested does not lead to sterility (Table 10-1).

STERILITY

Sterility indicates freedom from viable forms of microorganisms. It is an absolute state—there are no degrees of sterility. Either an object is sterile or it is not sterile. There may be billions of killed bacteria present, as in some vaccines, but if not a single viable bacterium is present the preparation is sterile. A preparation may be sterile and yet be harmful because of microbial products. Injection fluids, for example, may be sterile but contain fever- or shock-inducing pyrogens (endotoxins) that are derived from microorganisms.

-CIDE

A group of terms are constructed with the suffix *-cide* or *-cidal*, which means "to kill." A bactericide, a fungicide, or a sporicide is an agent or process that kills the type or form of organism named in the prefix.

-STASIS

A group of terms are constructed with the suffix *-static* or *-stasis*, which denotes inhibition or inac-

Table 10-1. Sterilization and Disinfection Terminology

Noun					
Process	Agent	State or condition	Verb	Adjective	Action, agent, or state
Sterilization	Sterilant (if a chemical)	Sterility	Sterilize	Sterile, sterilized	Completely destroys or removes all microorganisms; renders treated microorganisms permanently incapable of reproducing
	-cide			-cidal	Kills the form of microorganism named in the prefix: bactericide, sporicide, tuberculocide, fungicide, etc.
	-stat	-stasis		-static	Prevents multiplication of the type of microorganisms named in the prefix without necessarily killing it: bacteriostat, fungistat, etc.
		Sepsis		Septic	Breaks down living tissue by action of microorganisms; usually accompanied by acute inflammation, sometimes by toxicity
		Asepsis		Aseptic	"Without sepsis"; the absence or the exclusion of sepsis
	Antiseptic	Antisepsis	Antisepticize		"Against sepsis"; the application of nonchemotherapeutic agents to living tissue with the objective of at least preventing the multiplication of pathogenic microorganisms (does not ordinarily include spores)
Disinfection	Disinfectant		Disinfect	Disinfected	Prevents infection; carried out with chemical agents (disinfectants) or by physical means (e.g., boiling, pasteurization) on inanimate objects with the objective of at least killing (with the exception of spores) all harmful forms (e.g., pathogens)

tivation but not killing. A number of antiseptics, dyes, and antibiotics are microbiostatic agents that arrest the growth or prevent the multiplication of microorganisms. They produce fungistasis, bacteriostasis, etc.

SEPSIS

The breakdown of living tissue by the action of microorganisms is known as *sepsis*. It is accompanied by an acute inflammatory reaction on the part of the host. Pus formation is a prominent feature of bacterial sepsis. Toxic microbial products may contribute to the septic process. The host has an overwhelming infection, toxemia, or both when sepsis exists.

ASEPSIS

The avoidance of sepsis or deterrence to the development of sepsis is called *asepsis*. The goal of asepsis is to prevent, eliminate, or confine infection. A distinction is made between medical asepsis and surgical asepsis. Medical asepsis calls for practices that are designed to keep objects, personnel, patients, and the environment as free as possible from the agents that cause infectious disease. It includes the proper handling of linens, food, and body excreta and the employment of housekeeping and personal hygienic practices that serve to control microorganisms. The aim of surgical asepsis is to prevent microorganisms from ever gaining access to the wounds and tissues of the patient. Toward that end the instruments, surgical drapes, dressings, gloves, sutures, and other items that come in contact with the patient are sterilized; the immediate environment is scrupulously sanitized; ventilation is controlled; medical personnel are outfitted with sterile caps, gowns, masks, and gloves after employing prescribed scrub techniques on their hands and forearms; and the population of microorganisms on the patient's tissues is reduced as far as possible by the application of suitable antiseptics. In general, aseptic procedures seek to prevent the introduction of microorganisms not already present and to reduce the numbers of those that are present.

ANTISEPSIS

Antisepsis is a process or state that is against sepsis, that prevents the development of sepsis. Antiseptics are applied to living tissue with the objective of preventing the multiplication of those vegetative microorganisms that are capable of producing sepsis. Because they are applied to living tissue, antiseptics are often milder agents than disinfectants. Antiseptics are expected to have at least a stasis effect on the microorganisms. In practice the term *antisepticize* is especially used in situations in which antiseptics are applied to cuts, abrasions, or lacerations, and to tissues before and after surgery.

DISINFECTION

Disinfection at the very least destroys harmful (pathogenic) microorganisms that are in the vegetative state. The expected action of a disinfectant or of disinfection depends on the purpose for which the agent or process is employed and the species or types of microorganisms that are likely to be present. The target microorganisms in the health fields, for example, are the microorganisms (in the vegetative form if they are bacteria) that can cause an infection, whereas in an industrial situation the target microorganisms are those that might spoil or alter a product. A disinfected object should not be expected to be sterile; it may harbor a multitude of viable nonpathogens and spores of pathogens. According to the strict interpretation of the term, disinfection is carried out on inanimate objects, not on living tissue. The skin and mucosa are antisepticized. The distinctions between disinfection and antisepsis are frequently not made in everyday usage. A number of antimicrobial chemicals are used both as disinfectants and as antiseptics, often in the same concentration. Although disinfection is

generally carried out with chemicals (the disinfectants), it is also achieved by boiling, pasteurization, and other physical antimicrobial procedures.

SANITIZATION

Sanitization is a process that reduces the number of microorganisms on inanimate objects and in the environment to a safe level as judged by public health standards.

DEGERMATION

The removal of transient microorganisms of the skin by mechanical and chemical means is known as *degermation*.

CHEMOTHERAPEUTIC AGENTS

Chemotherapeutic agents such as the antibiotics and the sulfa drugs are usually taken internally. Some are selectively toxic; that is, they interact with specific microbial molecules and structures rather than with the host's molecules. Disinfectants and antiseptics are, in contrast, general protoplasmic poisons that interact in a more or less nonspecific manner with any molecular constituents of protoplasm, human or microbial.

GENERAL CONSIDERATIONS

POPULATION UNKNOWN

The population of microorganisms present on an object to be treated is seldom known. The assumption is made, therefore, that the most resistant microbial forms are present—the bacterial endospores.

DETERMINATION OF LEVEL OF KILL

Rarely is there a chance in routine practice to determine the level of kill obtained by the antimicrobial procedure. Such a determination requires the opportunity to sample-culture the treated item, a laboratory procedure. The principal criterion of the death of a microorganism is its inability to multiply when placed in suitable cultural conditions.

VARIATIONS WITHIN A POPULATION

The microorganisms within a population that are subjected to an antimicrobial agent or process do not all die at once. They are in different metabolic states, different microenvironments, and different phases of the growth cycle. If the death rate of a pure culture population is plotted, for the most part a straight line is obtained, as if it were a monomolecular reaction. Thus, if 90 percent of a population dies within one minute, then 90 percent of the remaining 10 percent dies in the next minute, and 90 percent of the 1 percent dies in the next minute, and so on. It is the death of the population that is measured, not the death of individual microorganisms as such. This concept will be further explained later, under Comparison of Heat Tolerance Levels of Microorganisms.

PROBLEM MICROORGANISMS

Certain microorganisms or their life forms are particularly troublesome from the standpoint of sterilization and disinfection: bacterial endospores, the mycobacteria, certain viruses that cause hepatitis, and members of the bacterial genus *Pseudomonas*.

Bacterial Endospores

The bacterial endospore is the most resistant form of microbial life. Some soil spores have withstood as much as 16 hours of boiling. Bacterial spores are ubiquitous. If the population of microorganisms on an object is unknown, it is best to assume that bacterial spores are present. That being the case, rig-

orous treatment is required to ensure that sterility is achieved.

Mycobacteria

The mycobacteria, of which the bacterium causing tuberculosis is the most important, are noted for their resistance to the disinfectants, especially the aqueous disinfectants. The mycobacterial cell surface is hydrophobic because the cell wall is rich in wax-like lipids. The mycobacteria do, however, have about the same level of susceptibility to heat as do the other bacteria that are in the vegetative state.

Hepatitis Viruses

The hepatitis B virus (HBV) and the non-A, non-B (NANB) hepatitis viruses are regarded as problem agents because of the inapplicability of sterilizing measures to the situations in which transmission most frequently occurs and because the effectiveness of disinfectants on the viruses is largely unknown. The durability of the viruses, the infinitesimal quantities that can initiate infection, the commonness of contact with blood, and the carrier rate in the general population—all contribute to the control problems that accompany the disease. It has been difficult to assess the effectiveness of disinfectants on the viruses and to establish the parameters of time, concentration, and so forth that are required to inactivate the viruses because, except in a few nonhuman primates, the viruses have not been cultured in the laboratory.

Pseudomonas

There are many free-living members of *Pseudomonas*, a genus of gram-negative bacilli. The few species that are associated with human infection are regarded as opportunists. *P. aeruginosa*, which is found in the feces of about 5 percent of humans, is the species best known for its role as a pathogen in the debilitated and immunosuppressed hospital patient. Burned patients and patients with acute leukemia are especially susceptible to *Pseudomonas* infections. In hospitals pseudomonads have been detected in food, medications, respiratory apparatus, monitoring equipment, and the waste traps of sinks. *Pseudomonas* can multiply even in some antiseptic solutions.

FACTORS THAT AFFECT THE ACTIVITY OF ANTIMICROBIAL AGENTS AND PROCEDURES

A variety of conditions have a bearing on the rate and the level of microbial destruction. Among these conditions are soil, pH, time-temperature relationships, and moisture.

Interfering Matter

A major consideration in sterilization and disinfection procedures is that of interfering matter, also referred to as *soil*. In the health-related fields, the interfering matter is largely organic matter derived from the body: blood, saliva, perspiration, urine, feces. Films of lubricants are also a form of soil. These substances provide protection for microorganisms, particularly against disinfectants. Disinfectants interact as well with the body proteins as they do with the microbial proteins. It is a cardinal tenet in processing soiled instruments, syringes, hypodermic needles, and other such items that a necessary first step is proper cleansing to remove the interfering matter. Aesthetic considerations are an added reason.

It is most important to understand the nature of the soil in question. If it is known or suspected that a soiled article harbors virulent infectious agents, the item must first be decontaminated before cleansing is instituted. Disinfection or autoclaving, depending on the problem at hand, may be the first procedure that is required.

pH

It is well known in the food and beverage industries that acid foods and beverages can be processed at lower temperatures, to make them commercially "sterile," than can alkaline foods. A general guideline among food microbiologists is that if the pH is below 4.5 pressure sterilization is not necessary. Wines and beers, because of their acidity (and alcoholic content), are pasteurized at lower temperatures and for shorter periods than is milk. The ef-

fectiveness of iodine compounds decreases as the pH rises above 6.

pH played a major role in the spontaneous generation dispute. Pasteur in his studies had used yeast infusions as the culture medium, a slightly acidic medium. Pouchet, who disputed Pasteur's results and conclusions, used hay infusions, which give rise to alkaline cultural conditions. The decreased susceptibility of microorganisms under alkaline conditions and the presence of spores of *Bacillis subtilis*, the hay bacillus, in the hay infusion made it appear that Pasteur had drawn erroneous conclusions about spontaneous generation. Further studies helped to refine the procedures and to define the parameters for achieving sterility.

Other Environmental Factors

The time length of exposure, the temperature level, the nature of the menstruum (solvent medium) in which the microorganism exists, the presence or absence of moisture, the tonicity (osmotic pressure) of the solution, and the growth phase of the microorganism are among the many other factors that can have a bearing on the rate of kill and the level of kill that an antimicrobial procedure or agent produces. Time-temperature relationships and moisture are discussed under Physical Methods.

NO UNIVERSAL METHOD

No given sterilization or disinfection procedure is applicable to every situation. The biological or physical nature of the object being subjected to an antimicrobial procedure differs and dictates the mode to be employed. Heat is the most sure, practical, and widely applicable means of controlling microorganisms. In some situations, however, heat is not appropriate because of the thermolability or thermosensitivity of the object to be treated. What may be an excellent way to control microorganisms on an instrument may be totally inapplicable on the oral mucosa. Generally speaking, in the health-related fields as much overkill is provided as conditions will permit.

CARELESS HANDLING

All the care that is exercised in sterilizing an item may be to no avail if the subsequent handling unwittingly recontaminates the object. A sterile instrument, if placed after removal from its sterile protective container on a contaminated surface or if touched by the fingers on critical parts, is no longer sterile and should be reprocessed. Certain treated or subsequently contaminated items, such as culture media, milk, and sera, when placed under incubational conditions will support the growth of microorganisms that survived the antimicrobial treatment. A new population of microorganisms may generate in a short time.

EFFECTS ON BACTERIA AS THE PRINCIPAL CRITERIA

Most of what is known and written about sterilization and disinfection relates to the bacteria. Parasitic and mycotic agents are not as resistant as the bacterial spores. Any method, then, that is sporicidal for bacteria is likely to destroy the most hardy of the parasitic and mycotic forms. For the most part, viruses are as readily killed by heat as are the vegetative forms of bacteria.

PRINCIPAL MODES OF ACHIEVING STERILITY

The routinely used methods for obtaining sterility are autoclaving with saturated steam under pressure; elevated dry heat (hot air), as in baking; exposure to the gas ethylene oxide (ETO); and exposure to the heated, unsaturated vapors of a combination of chemicals in an apparatus called the *Chemiclave*. The Chemiclave (formerly called a Harvey Vapor Pressure Sterilizer) is used primarily by the dental profession. There is no sterilization system—nor a single disinfectant, for that matter—that is applicable to every situation. The types cited are those that are used routinely. This is not to say that other devices or chemical agents do not produce sterility. It is possible, for example, to ster-

ilize by using appropriate filters, by irradiation, or by using chemicals such as glutaraldehyde, but such means are applicable in limited situations.

PHYSICAL METHODS

Sterilization, disinfection, and preservation procedures are customarily divided into physical methods and chemical methods. Physical methods include moist and dry heat, cold, filtration, ultrasonics, and irradiation.

HEAT

Comparison of Heat Tolerance Levels of Microorganisms

It is useful to determine the heat susceptibility of a given microorganism so that the extent of treatment to be applied in a given situation is understood. Much of what is known about the heat tolerance of microorganisms has been discovered by the food-processing industry. That industry must weigh the probability of microbial survivors in its products against the processing time. Protracted periods of heating may affect the palatability, appearance, and nutritional value of products, yet the products must be safe for consumption. The pharmaceutical industry is similarly interested in providing an adequate margin of safety without alteration of the product. There are certain terms or expressions concerning thermal kill of microorganisms. Some of the same procedures that gave rise to those terms are applicable to other sterilization and disinfection determinations. These expressions of susceptibility perhaps have less meaning as they apply to the health fields because whenever possible overkill is employed. Nevertheless they do provide a baseline and an insight into the resistance levels of microbial species and types.

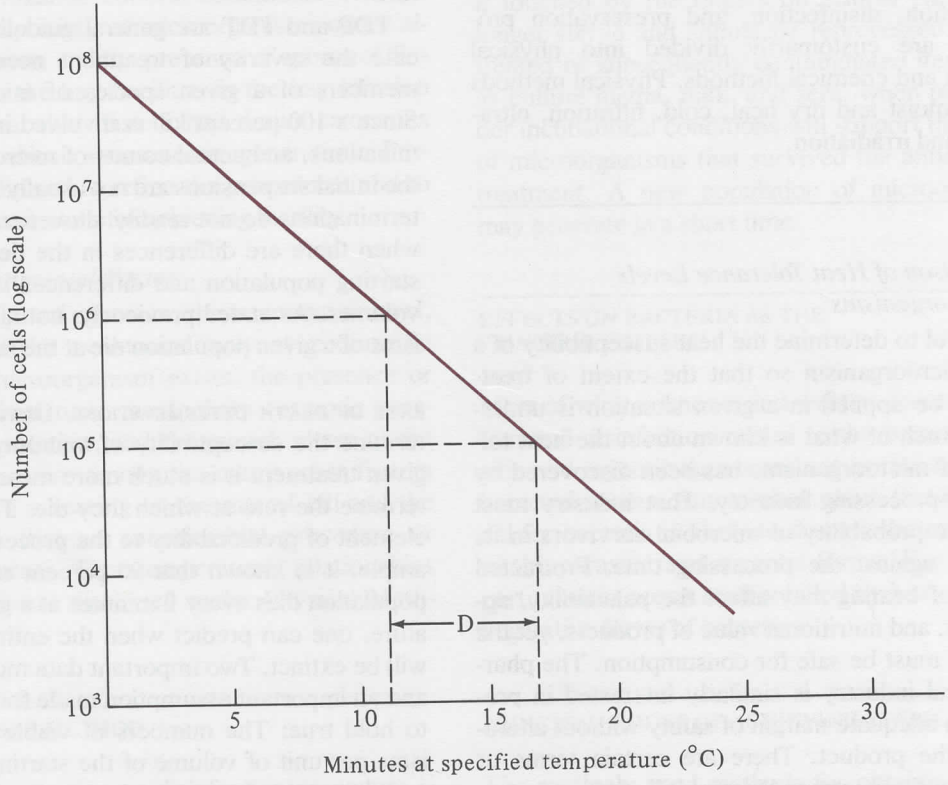
THERMAL DEATH POINT. The thermal death point (TDP) is that temperature at which all test microorganisms in a 24-hour broth culture at a pH of 7 are killed in 10 minutes.

THERMAL DEATH TIME. The thermal death time (TDT) is the time required to kill all microorganisms of a known population at a predetermined temperature under specified conditions. The determination is equivalent to asking, How long does it take to kill all the microorganisms in this suspension when they are held at this selected temperature?

TDP and TDT are general guidelines that indicate the severity of treatment necessary to kill members of a given species of microorganisms. Since a 100 percent kill is involved in these determinations, and actual counts of microorganisms of the initial suspensions are not usually made, the determinations do not readily allow for predictability when there are differences in the densities of the starting population and differences in the starting volumes. As stated previously, not all microorganisms of a given population die at the same instant.

RATE OF DEATH DETERMINATIONS. Obviously, to determine the susceptibility of microorganisms to a given treatment it is much more meaningful to determine the rate at which they die. This lends the element of predictability to the process. If, for example, it is known that 90 percent of the treated population dies every 2 minutes at a given temperature, one can predict when the entire population will be extinct. Two important data must be known and an important assumption made for this element to hold true: The numbers of viable microorganisms per unit of volume of the starting population and the total volume must be known. The assumption is that the death rate simulates a monomolecular chemical reaction—that is, that the kinetics of death are exponential. Thus, even though there are many types of molecules in microorganisms, the rate of kill makes it appear that only one type is the target of the killing agent, just as in a monomolecular chemical reaction only one type of chemical molecule is involved. If but one molecular species is involved, there should be a uniformity of reaction that is reflected in a straight line when the reaction is plotted on semilog paper. It is also therefore possible to make extrapolations from this graph. An important limitation must be noted. Theoretically,

Fig. 10-1. Determination of decimal reduction time (D). A sample of cells, for example, 1×10^7 cells per milliliter, is exposed to a given temperature over a specified period of time. The culture is measured at various times (minutes) for the number of microorganisms that have been killed. The data is plotted and a straight line is obtained. By drawing lines from the ordinate corresponding to one log unit (between 10^5 to 10^6 in the example) to the curve and then extrapolating to the abscissa, the decimal reduction time can be determined. In the above example, D is approximately 5 minutes, that is, it takes 5 minutes to reduce the population by a factor of 10. Theoretically, the population is reduced by a factor of 10 every five minutes.



all the microorganisms die in a uniform, predictable manner. The experience with many populations, however, has been that the last few microorganisms differ in susceptibility, which causes the slope of the graph to depart from the straight-line configuration. Extrapolations may be misleading and dangerous.

Decimal reduction time (D value) is the time in minutes that is required to kill 90 percent (1 log unit) of the population at a given temperature (Fig. 10-1).

Moist Heat

Most mesophilic bacteria in the vegetative form are killed by the comparatively low moist heat temperature of 60°C applied for 30 minutes. Vegetative forms of bacteria, yeasts, and molds are killed in 5 to 10 minutes at 80°C. Mold spores require 30 minutes at 80°C. The heat resistance of most viruses is similar to that of the vegetative forms of other microorganisms. Bacterial spores have a much greater thermoresistance. Studies show that it takes 330 minutes of boiling to kill the spores of

Clostridium botulinum, which causes botulism; 90 minutes to kill the spores of *C. tetani*, which causes tetanus; and 30 minutes to kill the spores of the gas gangrene organisms (*C. perfringens*). Lest total reliance be placed on these time and temperature quotations of the thermoresistance, it should be understood that different strains within the same species differ in their susceptibility and that experimental conditions vary.

The mode of action of moist heat is generally stated to be via the coagulation of proteins. Coagulation undoubtedly occurs when overkill conditions are attained, but thermal death probably results from subtler changes that take place before coagulation. Less drastic changes such as inactivation of enzymes, changes in nucleic acids, and cytoplasmic membrane alterations probably kill the microorganisms before coagulation occurs.

BOILING. Boiling cannot be relied on to achieve sterility within a practical period of time if the population of microorganisms is unknown. It is, however, an efficient means of physical disinfection. Vegetative forms of infectious agents are readily killed by boiling in a matter of minutes. At one time the tabletop boiler was a popular apparatus for "sterilizing" instruments, syringes, and hypodermic needles, but the method is not reliably sporicidal and therefore, cannot be depended on to ensure sterility. Moreover, instruments are dulled because of bumping and corrosion (rusting and electrolysis). Boiled items are easily recontaminated because they are not usually protectively packaged. The water on boiled items may become contaminated when instruments, syringes, and needles are "fished out."

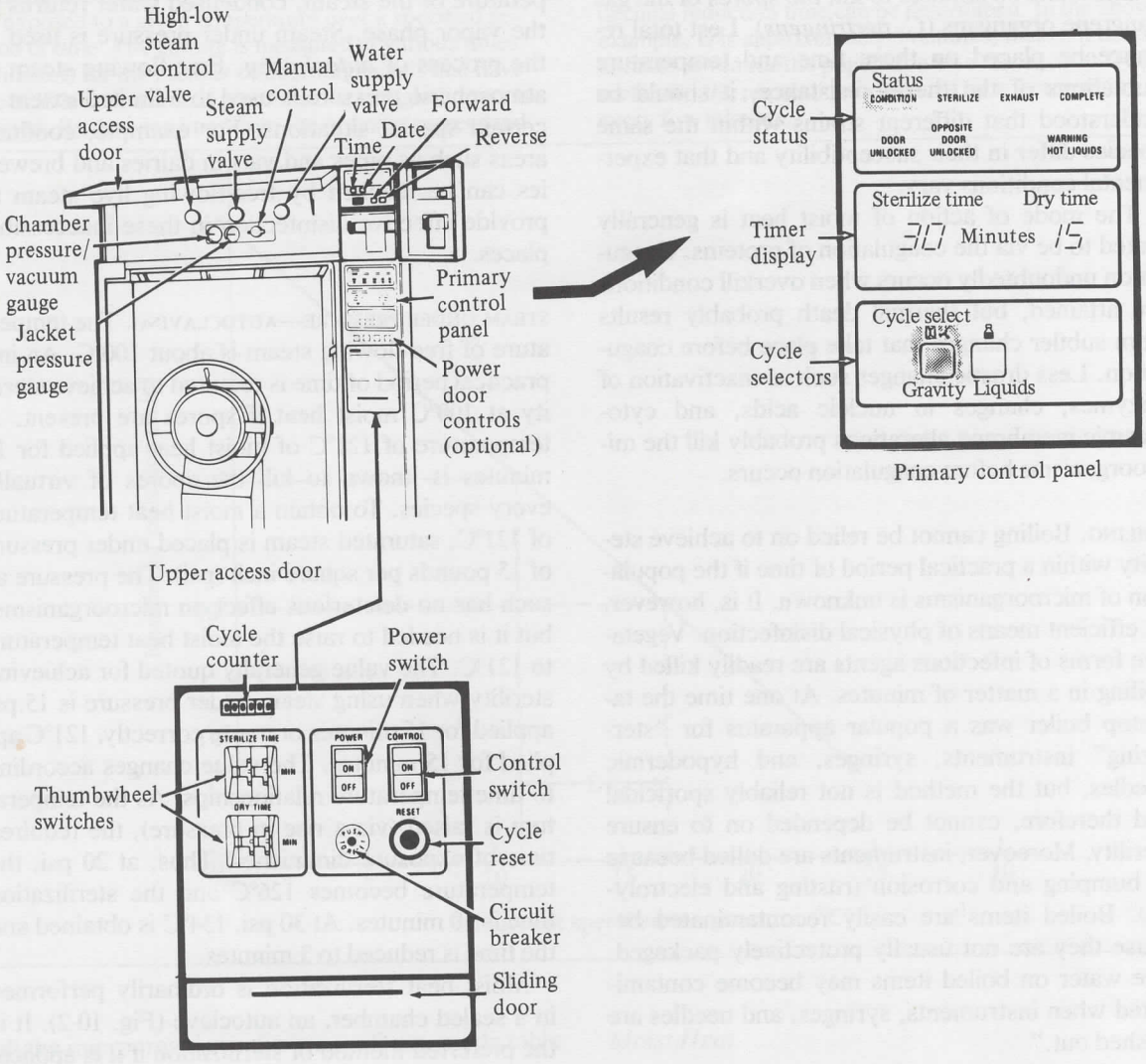
STEAM AT ATMOSPHERIC PRESSURE. Steam contains latent heat that is generated during vaporization. Steam at 100°C has 540 calories of heat as compared with the 80 calories of boiling water at that temperature. The latent heat is released when steam condenses on a colder surface. The condensation releases not only heat but moisture, which is necessary for protein coagulation to occur. The shrinking in volume of the steam during condensation creates a negative pressure, thereby drawing in more steam. As the heated object reaches the temperature of the steam, condensed water returns to the vapor phase. Steam under pressure is used in the process of *autoclaving*. Free-flowing steam at atmospheric pressure is used to a limited extent in certain special situations. For example, confined areas such as pipes and vats in dairies and breweries can be reached by free-flowing live steam to provide effective disinfection in these inaccessible places.

STEAM UNDER PRESSURE—AUTOCLAVING. The temperature of free-flowing steam is about 100°C. An impractical period of time is required to achieve sterility at 100°C moist heat if spores are present. A temperature of 121°C of moist heat applied for 15 minutes is known to kill the spores of virtually every species. To obtain a moist heat temperature of 121°C, saturated steam is placed under pressure of 15 pounds per square inch (psi). The pressure as such has no deleterious effect on microorganisms, but it is needed to raise the moist heat temperature to 121°C. The value generally quoted for achieving sterility when using steam under pressure is 15 psi applied for 15 minutes or, more correctly, 121°C applied for 15 minutes. The value changes according to time-temperature relationships. As the temperature is raised (via a rise in pressure), the required time of exposure diminishes. Thus, at 20 psi, the temperature becomes 126°C and the sterilization time is 10 minutes. At 30 psi, 134°C is obtained and the time is reduced to 3 minutes.

Moist heat sterilization is ordinarily performed in a sealed chamber, an autoclave (Fig. 10-2). It is the preferred method of sterilization if it is applicable. To request that something be autoclaved is tantamount to requesting that it be sterilized. There are two types of saturated steam autoclaves, the gravity air-removal type and the type in which a prevacuum is drawn to remove most of the air in the autoclave chamber. Unless otherwise stated, it is the gravity displacement type that is being referred to when autoclaving is described.

There are certain points to keep in mind when using an autoclave. The residual air must be totally

Fig. 10-2. Control panel of autoclave. (Courtesy American Sterilizer Co., Erie, PA.)



replaced by saturated steam because trapped air can prevent the proper temperature from being reached even though the pressure reading appears to indicate sterilizing conditions. Air may be trapped because of improper loading or because of an improperly operated autoclave. The admixture of air with steam in a double-walled (jacketed) autoclave, furthermore, can lead to superheated steam. Superheated steam acts like a dry hot gas rather than a vapor. The dry hot air takes up moisture readily. The latent heat and the moisture are spent upon the dry air instead of the surfaces of the items to be sterilized. Applying the minimum conditions of 121°C for 15 minutes does not necessarily produce a sterilized load. The type of load is a factor in determining the length of exposure (Table 10-2). Articles to be sterilized must not be packed tightly to allow for access of steam to the articles.

Sterilization Indicators. Since saturated steam autoclaving is the principal mode of achieving sterility it is fitting to describe sterilization indicators at this juncture. The two likely causes of sterilization failures are malfunctioning equipment and indequate treatment because of the size or nature or positioning of the load. The saturated steam autoclave is especially prone to malfunctions. The elevated heat, electrolysis, and the deposition of minerals contribute to the degradation and encrusting of valves, gauges, discharge lines, and so on. Loads differ in size and nature (e.g., hard-surfaced items vs. absorbent items), and these in turn affect the length of time that is required to attain sterilizing conditions in the innermost regions. Furthermore, the load may be incorrectly positioned, and sterilization indicators can alert operators to that failing.

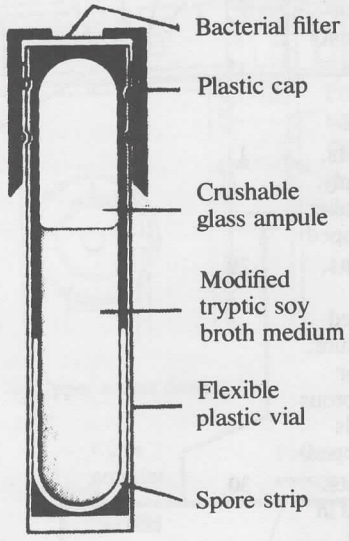
The commonly used indicators and checks are chemicals that undergo a color change and spores of selected bacterial species (biological monitors). Modern autoclaves indicate temperature in the chamber, record it, and automatically regulate temperature and pressure. Preferably such indicating and recording potentiometers should sense the conditions within the contents of the pack or load, but that arrangement is made with difficulty. The chemical indicators that exhibit a color change

Table 10-2. Minimum Sterilization Exposure Period—Wrapped and Unwrapped Goods, Gravity Cycle Only

Items	Autoclave setting (121°C) (250°F) (minutes)	Autoclave setting (132°C) (270°F) (minutes)
Dressings, wrapped in muslin or equivalent	30	15
Glassware, empty, inverted	15	3
Instruments, metal only, any number (unwrapped)	15	3
Instruments, metal, combined with suture, tubing or other porous materials (unwrapped)	20	10
Instruments, wrapped in double thickness muslin or equivalent	30	15
Linen packs (maximal size 12″ × 12″ × 12″, maximal weight 12 pounds)	30	—
Treatment trays, wrapped in muslin or equivalent	30	15
Utensils, unwrapped	15	3
Utensils, wrapped in muslin or equivalent	30	15

Source: Courtesy of American Sterilizer Co., Erie, PA.

Fig. 10-3. Biological sterilization indicator. The capsule contains a strip impregnated with bacterial spores, a pH indicator, and culture medium in a crushable ampule. After removal from the sterilizer the ampule is crushed, releasing the culture medium onto the spore strip. A yellow color after incubation indicates incomplete sterilization. (The capsule is part of the ATTEST system manufactured by the 3M Co., St. Paul, MN.)



have the chemical imprinted on tapes and wrappers for surface exposure indication and on tabs that are used for insertion into the interior of items. The former are less satisfactory for they merely indicate the conditions that prevailed on the package surface. Some of the color-change indicators have but a single area for change of color, while others have several sections, each of which indicates an increasing level of effect. The chemical color-change indicators offer the advantage of immediate readout, and they bear the evidence of exposure directly on or within the exposed article. The culture test (biological monitor) is regarded as the most reliable of the sterilization indicators. The major disadvantage is the requirement for an incubation period as long as 7 days. The spores of several species of bacteria with a known thermoresistance or a known resistance to ETO or to chemical vapors are commercially available in ampules or impregnated in filter paper strips (Fig. 10-3). The species whose spores are commonly used are *Bacillus stearothermophilus* for moist heat; *B. subtilis* var globigii for ETO, and *B. subtilis* var niger for dry heat. The Joint Commission on Accreditation of Hospitals and the Communicable Disease Center recommend that steam autoclaves and ETO sterilizers be tested at least once a week with appropriate spore indicators.

PASTEURIZATION. Pasteurization may be regarded as a physical method of achieving disinfection. Mild heat is used in pasteurization to destroy pathogenic vegetative forms while preserving the palatability of the liquid. In the beer and wine industries, the process is expected to kill contaminants and the microorganisms that produced the fermentation. The pasteurization of milk is expected to kill vegetative pathogens that are derived from infected cattle and from human and environmental contamination. Infectious diseases that are associated with transmission via raw milk include tuberculosis, brucellosis, streptococcal and *Campylobacter* infections, typhoid fever, dysentery, diphtheria, and Q fever. Q fever, which is caused by a species of *Rickettsia*, is creating some problems because it is transmitted to humans from cattle and is not uniformly susceptible to pasteurization.

With all microbial control processes that use heat, the higher the temperature the shorter the time the process must be applied. This point is nicely illustrated in the two milk pasteurization extremes. In the holding or batch method, milk in vessels such as vats is pasteurized at a temperature of 62.9°C applied for 30 minutes. In the flash method, thin films of milk stream over pipes or plates that are held at 71.6°C. An exposure of only 15 seconds is required to pasteurize in the latter high-temperature short-time pasteurization method.

Dry Heat

Thermosensitivity of biomolecules to dry heat is much less than to moist heat. Damaging alterations of proteins by dry heat are the result of oxidation, desiccation, and changes in osmotic pressure ow-

ing to the evaporation of moisture. Dry heat is slower and requires temperatures higher than those used in moist heat sterilization. A temperature of 170°C must be applied to anhydrous egg albumin to denature it. An aqueous solution of egg albumin is coagulated in 30 minutes at 56°C.

DRY HEAT STERILIZATION. Dry heat is customarily used at 160°C for at least 2 hours or 170°C for at least 1 hour. Dry heat temperatures below 140°C do not destroy spores within a reasonable period of time. Mechanically convected ovens are used to provide evenly distributed heat. Dry heat is the method of choice for sterilizing powders, oils, and thermostabile materials that are adversely affected by moisture. Packaging and arrangement of the load are as important as in autoclaving. Heat convected to the surfaces of the goods to be sterilized penetrates the items by conduction. Time required for penetration into the center of large packs or into tightly arranged items extends the time required to attain sterility.

INCINERATION. Discardable, combustible items that are contaminated are best disposed of by incineration. Proper handling of the contaminated objects before they are incinerated is an obviously necessary precaution to be taken. *Flaming* is a convenient way to kill microorganisms or to sterilize inexpensive heat-tolerant implements.

COLD

The effect of cold, which is not a method of sterilization, on microorganisms varies, depending on the species of microorganism and the level of cold at which the microorganisms are maintained. Refrigerator-level temperatures are used to preserve foods, biologicals, and various other substances and materials. Freezing-level temperatures, in some instances accompanied by drying of the product, are used to preserve cells, tissues, and cultures of microorganisms. Diagnostic laboratories preserve pure cultures as type cultures. Teaching institutions preserve cultures for laboratory and study demonstrations. Pharmaceutical houses and the food and beverage industries carefully preserve cultures that are utilized in the creation of their products.

Refrigeration

Ordinary refrigerator temperatures (2°C to 8°C) have a microbiostatic effect on microorganisms. Their metabolic rate is greatly reduced so that they neither reproduce nor shed products in this quiescent state. A few of the more delicate microorganisms—for example, the meningococcus, the etiological agent of epidemic meningitis—do not survive long at refrigerator temperatures. Refrigerator temperatures are viewed as being preservative of foods and not of the microorganisms. Contamination with psychrophilic microorganisms (those that grow at low temperatures) must be taken into consideration when intravenous fluids are being stored at refrigerator temperatures.

FILTRATION

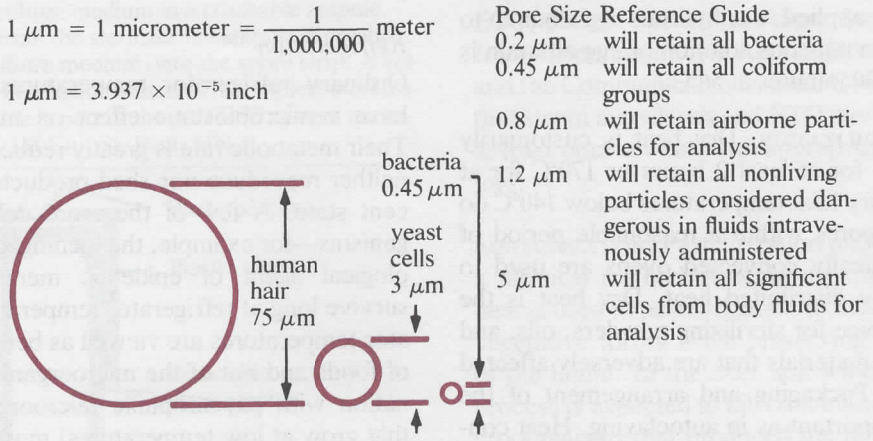
A number of porous materials are used to remove microorganisms from liquids and from the air. There are many applications of filters in microbiology: sterilization of liquids and air; separation of microorganisms of different sizes (viruses from bacteria); preparation of cell-free solutions of toxins, antigens, and enzymes; clarification of solutions; and determination of the size of viruses.

Bacteriological Depth Filters

Filters are available in a variety of pore sizes. The four principal types of filters that were used for many years are bacteriological depth filters: (1) Chamberland filters made of unglazed porcelain (kaolin); (2) Berkefeld filters made of diatomaceous earth; (3) Seitz filters made of compacted layers of asbestos; and (4) sintered glass filters made of fine, uniform particles of glass fused together in the form of a disk.

These filter types have been largely replaced by the popular and versatile membrane filters. The classic bacteriological depth filters have certain drawbacks compared to the newer membrane fil-

Fig. 10-4. Reference guide to pore size of membrane filters. (Courtesy Gelman Sciences, Inc., Ann Arbor, MI.)



ters. Because of their thickness and absorption, depth filters retain some of the fluid. They have a slower flow rate and tend to become clogged. Except for the discardable asbestos of the Seitz filter, they require cumbersome cleaning and sterilization procedures to ready them for reuse.

Membrane Filters

Membrane filters are paper-thin elements composed of cellulose esters and other polymeric materials. Unlike the tortuous path provided by the depth filter matrix, their sieve-like nature facilitates filtration. The pores are comparatively uniform in size, and the flow rate is much superior to that of the depth filters because of the numerous pore openings. The pore sizes range down to sizes that retain the smallest viruses and some of the larger protein molecules (Fig. 10-4). They are disposable, can be autoclaved (they can also be purchased as single-use, sterilized devices), and are compatible with many chemicals. Some can be made transparent so that the materials collected on the surface can be stained and examined under the microscope. Body fluids can be run through a filter and the filter then aseptically placed on an agar medium. Organisms present in the fluid—for example, in a urine specimen—grow on the filter surface and can be enumerated. Filters that are used for enumeration are often marked off in grids to facilitate counting (Fig. 10-5).

Membrane filters have many applications in science and industry. The cytopathologist concentrates cells obtained from body fluids such as gastric washings, stains them, and examines the preparations for neoplastic cells. Artificial lungs that are used during open-heart surgery use membrane devices to remove CO_2 and to oxygenate the blood.

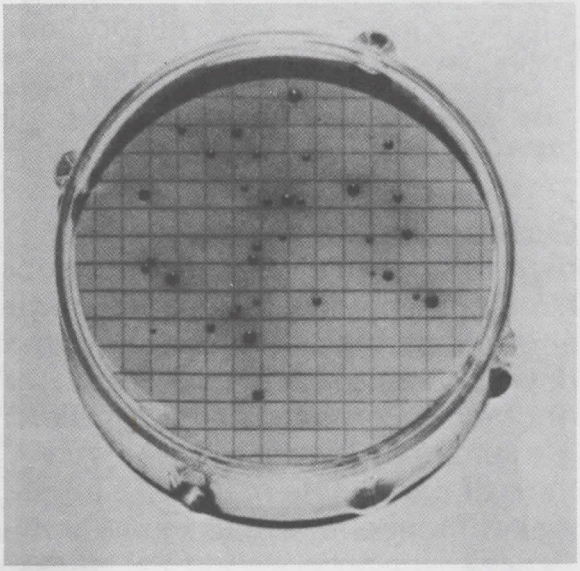
High-Efficiency Particulate Air Filters

A popular application of filters is in air control systems for the purpose of removing particles, including microorganisms. High-efficiency particulate air filters (HEPA filters) remove particles as small as 0.3 μm with 99.97 percent efficiency. They are used in "clean room" installations (where the intent is to avoid the alighting of particles on delicate instruments) and in biohazard hoods.

ULTRASONICS

High-frequency sound waves, above the human auditory level, have several applications in microbiology and in the health-related fields. They are used

Fig. 10-5. Bacterial colonies on membrane filter with grid. Bacteria collected on a membrane filter develop into colonies when the membrane is placed on an agar medium. The grid facilitates enumeration of the bacteria that were present in the filtered specimen. (Courtesy Gelman Sciences, Inc., Ann Arbor, MI.)



in the laboratory for cell disintegration and in sterilization and disinfection for cleansing purposes.

High-frequency electrical energy is converted into mechanical energy by metallic or piezoelectric transducers. The mechanical energy is in the form of high-frequency sound wave vibrations.

Ultrasonic Cleaners

The prime interest in ultrasonics in relation to control of microorganisms is in the ultrasonic cleaners (Fig. 10-6). They are used as a presterilization mode to remove interfering matter. They consist of tanks filled with liquids, through which sound waves are propelled. Ultrasonic cleaners are very efficient in removing organic soil from instruments and glassware. They surpass manual brush cleaning of instruments. Immersion of an instrument for about 15 seconds removes most of the soil, including that in serrated and hinged areas, where brushes do not readily reach.

Mechanism of Action

The removal of soil by ultrasonic cleaners is largely due to the phenomenon of cavitation. As high-intensity sound waves traverse the cleaning liquid, myriads of minute bubbles are generated. The bubbles increase in size until they collapse violently. The collapse creates a suctioning action (cavitation) that removes the soil. The "cold boiling" of the liquid also contributes a mechanical action that serves to dislodge debris.

Other Ultrasound Applications

There are numerous other applications of ultrasound apart from those in microbiology. Ultrasonic devices are used for noninvasive scanning of body organs, a procedure that can take the place of exploratory surgery and x-rays. They are used for tissue repair, for healing eyes, for smog control, for creating aerosols, as intruder alarms, for gauging thicknesses, for mixing and creating emulsions, for defoaming, and for plastic sealing.

RADIATION

Electromagnetic energy affects cells in varying ways, depending on the wavelength, intensity, and duration of the energy. The absorbed energy may produce fluorescence (infrared), heat (infrared and ultraviolet), and photochemical changes in which the absorbing molecules of the cell are internally rearranged and decomposed (ultraviolet and ionizing radiation).

Ultraviolet Radiation

Absorbed ultraviolet (UV) rays bring about photochemical reactions, particularly in DNA. The important changes are in the form of mutations and a microbicidal effect. The major effect at the molecular level is the formation of pyrimidine dimers within the DNA strand. Adjacent pyrimidines, thymine especially, form lethal carbon-to-carbon linkages that affect normal base pairing (see Chap. 5).

The bactericidal range of UV rays is between 2400 and 2800 angstroms (Å). The optimum bactericidal effect is around 2600 Å, which coincides with the absorption peak of DNA for UV rays.

Fig. 10-6. Portable ultrasonic cleaner and rinser-dryer. After ultrasonic cleaning the rinser-dryer removes detergent residues and dries the load. (Courtesy Sybron Corp., Medical Products Division, Rochester, NY.)

IRRADIATION OF ROOM AIR. The practical application of UV radiation in the control of microorganisms is primarily that of reducing the population of microorganisms in the air. UV lamps are referred to as *germicidal lamps*. Most germicidal lamps are low-pressure mercury-vapor lamps that generate UV rays with a predominant wavelength of 2537 A. Low-pressure mercury-vapor lamps are chosen over the more effective high-pressure mercury-vapor lamps for practical reasons; high-pressure lamps are more expensive and are explosive.

There are UV barrier installations by which incoming ducted air of buildings is irradiated with germicidal lamps. The air and room surfaces of hospital rooms, operating rooms, entryways, nurseries, gymnasiums, and cafeterias can be irradiated by louvered or shielded ceiling fixtures. The fixtures are designed in such a manner that the UV energy is reflected through a maximum distance without striking the occupants of the room. Warmed air normally rises to the ceiling, carrying with it the microorganism-bearing droplet nuclei (minute particles of mucus that are aerosolized during talking, sneezing, and coughing).

Other microbial control applications include the sterilization of biologicals (vaccines, sera, toxins)

and product protection. Some sanitation procedures of food packaging and preparation industries is accomplished with germicidal lamps. Nonedible products such as instruments, paper goods, industrial liquids, and the contents of storage cabinets are maintained, sanitized, or sterilized with UV lamps.

LIMITATIONS OF ULTRAVIOLET RADIATION. There are two major drawbacks associated with UV irradiation when used in the control of microorganisms. UV rays have low penetrability. Organisms must be rather directly exposed to the incident rays. Organisms within solids or organisms protected or shielded by any covering material such as ordinary glass, paper, or textiles remain unaffected. Precautions must be taken to avoid prolonged exposure of humans to intense UV radiation. Overexposure to UV lamp irradiation causes skin erythema and conjunctivitis. There have been cases in which a number of persons who attended or played basketball games in gymnasiums suffered conjunctivitis, which gave the sensation of "sand in the eyes" and was accompanied by itching, burning, tearing, and swollen eyelids. Investigations traced the cause of the conjunctivitis to overhead mercury lamps whose outer protective globes or casings had been damaged by high-flying basketballs.

Ionizing Radiation

Electromagnetic rays of shorter wavelength are capable of dislodging orbital electrons from atoms. When directed at microorganisms, these rays have profound effects. Short-wavelength electromagnetic rays include x-rays, which are produced by electron accelerators, and gamma rays, which are produced by decay of radioactive substances. When passing through matter, the energy of the rays causes the ejection of electrons, leaving the atoms with a positive charge. The dislodged electrons attach to other atoms to render them electronegative.

Although the precise mechanism is not clearly understood, ionizing radiation is believed to affect living matter in one of two ways. According to the target theory, atoms of critical biomolecules, particularly DNA, are affected directly by the ionizing rays, and irreversible damage results. The other effect is an indirect one exercised through the water molecules of the cell. Ionization of cell water produces free hydroxyl radicals, which serve as powerful oxidizing agents, and hydrogen radicals, which serve as reducing agents. The radicals in turn cause secondary reactions in sensitive biomolecules, particularly in DNA.

ADVANTAGES AND DISADVANTAGES. Ionizing radiation when used in sterilizing offers the advantages of being unaffected by heat and moisture, of having great penetrability (gamma rays, for example, penetrate 2 feet of water), and of allowing prepackaging and sealing of the items to be sterilized. The disadvantages are the special expensive equipment installations, the required safety measures, and the effects on the materials that are exposed. The effects on materials include loss of tensile strength in fabrics, discoloration, chemical changes in certain pharmaceuticals, and organoleptic (e.g., taste) changes in food.

APPLICATIONS. Ionizing radiations are especially useful for sterilizing pharmaceuticals and disposable medical, dental, and laboratory supplies, such as plastic syringes, catheters, surgical gloves, blood transfusion sets, suturing materials, and the many other plastic supplies and devices used in the health-related field. Radiopreservation of food has been researched for 40 years, but industry regards it as not economical. It has been legal in the United States since 1963 to apply low-dose radiation to wheat and wheat flour to kill insects (disinfest), and to market-bound potatoes to prevent sprouting. Irradiation of food could help to alleviate world hunger because it is estimated that food spoilage in countries with humid climates and inadequate storage facilities runs as high as 25 to 30 percent. The armed forces have extensively investigated the radiosterilization of perishable foods. Spoilage could be reduced during shipping and storage without expensive and cumbersome refrigeration installations. However, irradiation, while being able to sterilize the food, affects its palatability, appearance, and nutritional value.

CHEMICAL METHODS

Chemical agents used for the control of microorganisms on body surfaces and on inanimate objects are grouped under the general heading of disinfectants or germicides. They include antiseptics and sanitizing, degerming, and disinfecting agents. In certain contexts, then, the word *disinfectant* or *germicide* represents all chemical agents that are used in sterilization or disinfection.

There are a number of situations in which it is not possible to achieve sterility or in which sterility is not really necessary. In many instances all that is required is to reduce the population of microorganisms to a safe level or to rid an object of the habitually pathogenic vegetative forms. Microorganisms are ubiquitous; they are a part of our everyday lives. The normal healthy body is able to keep the majority of them in check.

Sterility can be attained with certain chemicals when they are used under prescribed conditions. Such chemicals are referred to as *chemosterilizers*. They include ethylene oxide (ETO), betapropiolactone, some phenolics and halogens, strong acids and alkalies, formaldehyde and glutaraldehyde, and peracids. Situations in which chemosterilizers are applicable (with the exception of ETO) are very limited.

QUALITIES OF A UNIVERSAL DISINFECTANT

Just as no single physical method is applicable to every sterilization or disinfection problem, no one chemical agent is able to contend with the many variables that are part of differing control problems. A universal disinfectant would have the following qualities:

1. It would be able to destroy all forms of microorganisms within a practical period of time.
2. Soil would not interfere with its actions. Most disinfectants interact as well with the reactive chemical groups of organic matter such as body proteins as with the chemical groups of microorganisms.
3. It would be nonirritating to tissues, nonallergenic, and nontoxic.
4. It would be noncorrosive, nondiscoloring, nondegradative; it would leave no unsightly or toxic residues on inanimate objects.
5. It would make effective contact. Wettability and penetrability might be part of this quality.
6. It would be soluble in readily available solvents, preferably in water.
7. It would be chemically stable; neither it nor the solvent would evaporate and thus lead to a too-concentrated solution damaging to tissues or objects.
8. Its effective range would not be easily bypassed as a result of reasonable diluting factors.
9. It would not have a disagreeable odor, leave stains, or be inordinately expensive.

No disinfectant meets all these qualifications. There are no "all-purpose" disinfectants. They must be chosen on the basis of their intrinsic properties and on the basis of the job at hand.

MODE OF ACTION

The mechanisms of action of chemical disinfectants are complex and more or less nonspecific. Seldom do disinfecting actions affect only one type of microbial structure or molecule. The same disinfectant, depending on its concentration, the length of time it is allowed to act, and other factors, may be found to have several damaging effects on the microorganism. It may act on the lipid portion of the cell membrane, it may oxidize or reduce an important functional group of an enzyme, and it may coagulate microbial proteins. This nonspecificity is also related to the fact that disinfectants are not selectively toxic, as are some chemotherapeutic agents. Despite the nonspecificity and nonselectivity, it is possible to speak in general terms of the mode of action of disinfectants and to cite what is believed to be the principal mode of action of the various chemical disinfectant groups.

Cell Membrane Injury

The cell membrane, because of its lipid and enzyme constituents and because of its accessibility, is the target of action of many disinfectants. Agents that contain lipophilic groups—for example, the long-chain aliphatic groups of the cationic detergents—affect the bacterial cell membrane. They alter the permeability of the cell, or they cause cell leakage and lysis.

Coagulation and Denaturation

Some disinfectants have a strong coagulant action. Cell proteins are normally dispersed within the cell in the colloidal state. When the finely divided cellular particles coagulate, they become nonfunctional. Some agents denature proteins by causing the unfolding of polypeptide chains. Conformational changes result from the coiling and looping as the unfolded chains irregularly form random bonds.

Interactions with Functional Groups of Proteins

The functionally reactive chemical groups of organic molecules include the carboxyl (–COOH), hydroxyl (–OH), amino ($–NH_2$), and sulfhydryl (–SH) groups. Heavy metals and alkylating agents such as ETO and formaldehyde readily react with these groups. If the groups are binding sites of enzymes, the enzyme is unable to interact normally with its specific substrate, and catalysis is inhibited. Interfering organic matter reduces the effectiveness of these disinfectants.

Oxidation

Many proteins have side chains that contain the vital sulfhydryl group (–SH). This group is important for maintaining the conformation of protein molecules as well as the catalytic activity of many enzymes. Enzymes may not function properly if the –SH group is in the oxidized state. Oxidizing agents such as chlorine and hydrogen peroxide convert –SH groups to the oxidized S–S form.

DESIGN OF DISINFECTANTS

There are more than 8000 registered disinfectants on the market, and thousands of other compounds have been investigated for their antimicrobial effects. Many of these compounds were not originally discovered as disinfectants but are chemical homologues of known active compounds. It is a standard procedure of the pharmaceutical and chemical industries to manipulate an active compound chemically with the objective of improving its properties, for example, microbial toxicity, solubility, or binding to target sites. If possible and practicable, the molecule is synthesized in the laboratory. The promising leads are exploited by removal of a functional group and addition of the same or different groups or combinations elsewhere to the core or carrier molecule. The removal or insertion of the functional groups may bring about changes in the bonding to the target molecule. The bonding component changes may be in the form of disruption of bonds or formation of new bonds. Or, instead of a change in bonding, there may be a change in the steric configuration—that is, a dimensional change in the disinfectant or drug molecule's structure that improves the "fit" with the receptor molecule.

Among the disinfectants the phenol-related compounds, collectively called the *phenolics*, exemplify the principle of the structural relationships of molecules to their activity. One way to increase the effectiveness of phenol derivatives is to make the compounds more lipophilic. Long-chain hydrocarbons up to a certain length are very fat soluble. The phenol coefficient (see Phenol Coefficient Test later in this chapter) increases with an increase in alkyl chain length of the substituent group of phenol (Table 10-3). The addition of a halogen in certain positions on phenols or alkylphenols further enhances the activity.

EVALUATION OF DISINFECTANTS

The U.S. Department of Agriculture (USDA) regulates interstate trade of antimicrobial agents that

Table 10-3. Microbicidal Action of Phenol Derivatives

Substituting radical	Phenol coefficient (37°C)			
	S. typhosa	*S. aureus*	*M. tuberculosis*	*C. albicans*
None	1.0	1.0	1.0	1.0
2-Methyl	2.3	2.3	2.0	2.0
3-Methyl	2.3	2.3	2.0	2.0
4-Methyl	2.3	2.3	2.0	2.0
4-Ethyl	6.3	6.3	6.7	7.8
2,4-Dimethyl	5.0	4.4	4.0	5.0
2,5-Dimethyl	5.0	4.4	4.0	4.0
3,4-Dimethyl	5.0	3.8	4.0	4.0
2,6-Dimethyl	3.8	4.4	4.0	3.5
4-*n*-Propyl	18.3	16.3	17.8	17.8
4-*n*-Butyl	46.7	43.7	44.4	44.4
4-*n*-Amyl	53.3	125.0	133.0	156.0
4-*t*-Amyl	30.0	93.8	111.1	100.0
4-*n*-Hexyl	33.3	313.0	389.0	333.0
4-*n*-Heptyl	(16.7)*	625.0	667.0	556.0

*Figure is approximate.
Source: From J. W. Baker, I. Schumacher, and D. P. Roman, in A. Burger (Ed.), *Medicinal Chemistry* (3rd ed.). New York: Wiley-Interscience, 1970.

are used on inanimate objects—namely, the disinfectants. Products used on humans and animals are categorized as drugs and are therefore subject to Food and Drug Administration (FDA) regulations. Antiseptics are in the latter category. It is apparent that the distinctions between disinfectants and antiseptics that were made under Terminology at the beginning of the chapter are important when it comes to responsibility for regulation. Basic methods of evaluation of the two categories are the same for the most part, except that the testing of antiseptics poses the additional problem that in-use testing should be carried out on living tissue.

Initial Testing

The chief objectives of initial testing are to determine (1) the intrinsic capabilities of the antimicrobial agent and (2) the concentrations to be considered for practical application. Determining the intrinsic capabilities means finding out whether the test chemical has the inherent ability under controlled laboratory conditions to kill or prevent the multiplication of certain representative test microorganisms when there is direct access to the microorganisms. The first requirement is that the test disinfectant has an antimicrobial effect in the absence of any interfering factors that might occur in practical situations. Initial testing, then, is a *first-performance* or *screening test*.

In-Use Testing

Once the intrinsic value has been established, the test disinfectant should be tried under in-use conditions or under circumstances that simulate in-use conditions. Numerous factors must be taken into account in the evaluations. Consider, for example, the reason for neutralizing or removing the test disinfectant after selected intervals of exposure of the test microorganisms. Many of the earlier investigations did not incorporate this precaution. In suspension tests, dilutions of the disinfectant are added to the suspension of microorganisms, and samples are taken periodically. The samples contain a carry-over of the disinfectant when they are

inoculated into sterile media for determining the presence of survivors. Certain disinfectants, if their effective concentration is not bypassed, continue to act in the subculture, making it appear that the disinfectant killed the test organisms in the selected test interval. The carry-over disinfectant must be neutralized or removed to avoid erroneous conclusions. Neutralizing or quenching noninterfering chemicals can be added, or the test cells can be collected on membrane filters and washed.

The Phenol Coefficient Test

The best-known of the disinfectant evaluation tests is the phenol coefficient test. Dilutions of pure phenol serve as the standard of comparison. It is a test of long standing that has the official approbation of the USDA. It can be viewed as a first-performance, initial test.

PHENOL COEFFICIENT DETERMINATIONS. The phenol coefficient (PC) is a number that is arrived at by comparison of the activity of a standard phenol dilution to the activity of a dilution of the test disinfectant. The activity is judged against selected laboratory strains of microorganisms under specified laboratory conditions. For establishing the phenol coefficient, that dilution is chosen that does not kill in 5 minutes but kills in 10 minutes as observed in the subculture. The reciprocals of the dilutions of phenol and the test chemical are then compared, and the PC is determined from that result. Thus, if the end point for phenol were 1:90 and that of the test disinfectant 1:540, the PC of the test compound would be 6.

$$\text{PC} = \frac{\text{dilution of test disinfectant}}{\text{dilution of phenol}} = \frac{540}{90} = 6$$

The PC no longer must appear on the labels of disinfectants. It was commercially exploited, and to the uninformed it had a magical meaning. Too often the number is virtually meaningless unless the compound is used on pure cultures, an unlikely application in most situations. As stated previously, its function is that of a first-performance test that determines the intrinsic value of the test disinfectant. Tests under in-use conditions are the true evaluative guides.

CLASSES OF DISINFECTANTS

We cannot describe the properties, applications, advantages, and disadvantages of the thousands of disinfectants on the market. It is customary to divide the disinfectants into several major classes based on chemical relatedness: phenol and phenolics, alcohols, halogens, surfactants, alkylating agents, and heavy metals. Brief general commentaries are made about the salient features of the members of each class.

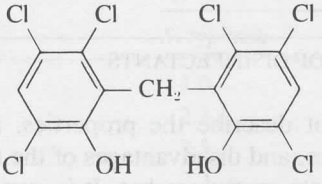
Phenol and Phenolics

Phenol, formerly called *carbolic acid*, figured in a medical classic. Lister used sprays of phenol and phenol-soaked dressings to markedly lessen infections in surgical and traumatic wounds. Dilutions of pure phenol are not used today as antiseptics because of phenol's irritant and anesthetizing qualities, nor are they used as disinfectants because of the disagreeable odor.

Phenol is the parent compound of a large number of disinfectants referred to as *phenolics, phenol derivatives, phenol-related compounds*, and *phenol homologues*. Since phenol and phenolics are among the more active compounds in the presence of organic matter, they are suitable agents for disinfecting saliva, feces, and similar matter. They are stable and persist on surfaces for long periods after application. Dried residues again become active when rehydrated. They thus have a combination of desirable features lacking in many disinfectants: They are tuberculocidal, and they are active in the presence of organic matter.

HEXACHLOROPHENE. Chemically, hexachlorophene (HCP) is a bisphenol; that is, two molecules of phenol are joined together (Fig. 10-7). Three chlorine atoms are positioned on each phenol molecule, for a total of six (*hexa* is the Greek word for six). Originally called G-11, hexachlorophene has been incorporated into soaps and lotions that are used

Fig. 10-7. Hexachlorophene.

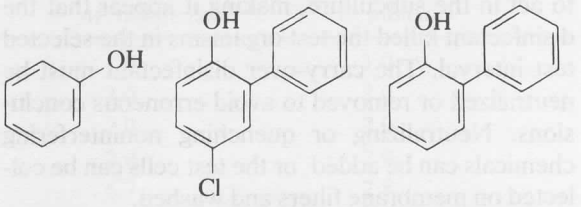


for surgical scrubs and hospital microbial control procedures. It has also been used in cosmetic soaps and deodorants and feminine hygiene sprays. A bacteriostatic agent, it has the quality of persisting on the skin, thereby providing an ongoing low level of antimicrobial activity. Because of the feature of accumulation and persistence on the skin and the ability to reduce the skin bacteria, it was highly recommended for use in clinical and hospital practice and was popular in hospital nurseries for the prophylactic bathing of infants. It was especially valuable in controlling hospital nursery staphylococcal infections, a continually worrisome problem of the hospital nursery.

Since 1972 there has been a change in the use of hexachlorophene. Shown to be toxic, it has become restricted to a prescription item. It is absorbed through the intact skin. According to experimental studies, it causes gross brain damage in rats. Studies of newborn infants who received three or more daily baths in 3% HCP for more than 3 days revealed that a percentage of the premature infants suffered brain damage. The FDA has directed that hospitals restrict HCP to prescription use. It is not to be employed in hospital nurseries unless a staphylococcal epidemic is flourishing.

OTHER PHENOLICS. Other effective phenol-related compounds are chlorophene and orthophenylphenols (Fig. 10-8). Commercial preparations include the products Amerse, O'Syl, Dettol, and Amphyl. Cresols and xylenols are phenolic-related compounds that are coal tar derivatives. They are generally used for sanitation.

Fig. 10-8. Phenol and two popular phenol-related compounds.



Alcohols

The value of alcohols as antimicrobial agents was underrated for years because of misleading studies conducted earlier in the century. Alcohols are bactericidal and fungicidal but not sporicidal. Enveloped viruses are susceptible to the alcohols because of the lipid components of the viral envelope. The mode of action of alcohols is primarily that of denaturation of proteins. The solvent action on lipids is regarded as another mode of action. Ethyl and isopropyl alcohols are the only alcohols that are extensively used, though longer-chain alcohols are more effective. The latter are avoided for the practical reasons that they have disagreeable odors, are not as readily available, and decrease in solubility as the molecular weight increases. Ethyl and isopropyl alcohols are about equally effective. Isopropyl alcohol is less volatile and does not have the legal restrictions placed on it that ethyl alcohol does. The recommended optimum concentration is 70% even though concentrations from 60 to 95% seem to kill as fast. Absolute alcohol is less effective than the aqueous solutions, which contributes to the belief that alcohols act by denaturation of proteins, a process requiring water. A disadvantage of the alcohols is that the effective concentration is easily bypassed. Once the concentration falls below 45%, the antimicrobial activity is slow and uncertain.

Halogens

Most disinfectants categorized as halogens are inorganic halogen compounds. The mode of action is

due to oxidation and to direct halogenation of proteins. The commonly used halogens are chlorine and iodine. All halogens are, furthermore, important as substituents in other disinfectants, in which they play a contributory antimicrobial role as already described under Design of Disinfectants. Chlorine is especially important as a substituent in other disinfectants.

CHLORINE. Elemental chlorine and inorganic chlorine compounds are employed principally in sanitation (sanitation refers to the reduction of a population of microorganisms to a level that is regarded safe as judged by public health requirements), purification, and disinfection. Elemental chlorine is used for water purification. Hypochlorites and other chlorine-releasing compounds serve as bleaching agents and as sanitizing agents in dairies, abattoirs, and swimming pools, and in housekeeping. Organic matter interferes with the antimicrobial activity of the chlorine compounds to a considerable extent.

IODINE. Iodine compounds currently are the most popular for wound and skin antisepsis and for preoperative skin antisepsis. They are also used as general disinfectants for thermometers and surgical appliances and for purification of water.

Tinctures of Iodine. The early popular iodine formulations were tinctures of iodine. Molecular iodine is not readily soluble in aqueous solution. It is therefore usually dissolved in alcohol (hydroalcoholic solutions). Solubility of the tinctures of iodine is increased by the addition of iodides (NaI and KI). The ready evaporation of alcohol creates problems because strong iodine concentrations can cause tissue necrosis. Tinctures of iodine leave noticeable stains, and those that are prepared with iodides elicit allergies in some individuals. Tincture of iodine formulations contain differing percentages of elemental iodine and of alcohol. One, five, and seven percent tincture formulations destroy 90 percent of skin bacteria in 90, 60, and 15 seconds, respectively.

Iodophors. The disadvantages of the tinctures were largely overcome by the development of a group of compounds collectively called the *iodophors*. Iodophors are composed of complexes of iodine and surface-active organic carrier molecules. The complexed iodine is released gradually from the carrier molecule. The activity is not as great as with the tinctures because of the lowered available iodine content. Iodophors are nonstaining, nonallergenic, water soluble, and relatively nonirritating and thus have an advantage over the inorganic iodine formulations. Proprietary iodophors include Wescodyne, Iosan, Virac, Hi-Sine, Betadine, Surgidine, and Prepodyne.

Surfactants

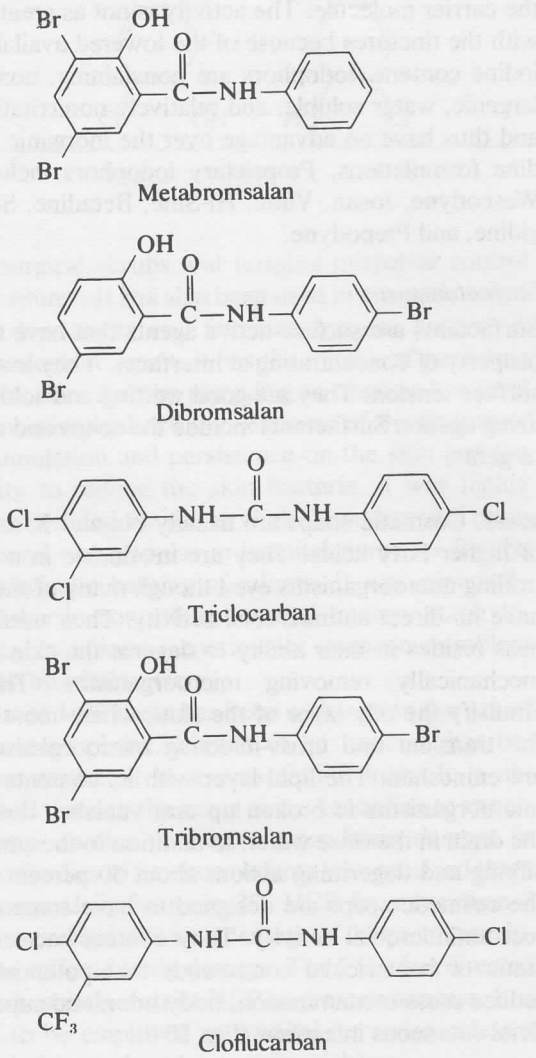
Surfactants are surface-active agents that have the property of concentrating at interfaces. They lower surface tension. They are good wetting and solubilizing agents. Surfactants include the soaps and detergents.

SOAPS. Cosmetic soaps are usually Na and K salts of higher fatty acids. They are invaluable in controlling microorganisms even though many of them have no direct antimicrobial activity. Their usefulness resides in their ability to degerm the skin by mechanically removing microorganisms. They emulsify the oily layer of the skin, where most of the transient and cross-infecting microorganisms are enmeshed. The lipid layer with its contents of microorganisms is broken up and vanishes down the drain in the rinse water. In addition to the emulsifying and degerming action, about 50 percent of the cosmetic soaps are designed to have some direct antimicrobial activity. They contain bacteriostatic or bactericidal compounds that potentially reduce cross contamination, body odor, and superficial cutaneous infections (Fig. 10-9).

DETERGENTS. Detergents are synthetic surfactant cleansing agents. They are regarded as superior to the soaps as cleansing agents because they do not form precipitates and deposits with water minerals as the soaps do. They are divided into the anionic, cationic, and nonionic detergents on the basis of the polarity and activity of their polar groups.

Anionic Detergents. The cleansing properties

Fig. 10-9. Bacteriostatic agents used in soaps such as Lifebuoy, Gamophen, Dial, Palmolive Gold, and Phase III.



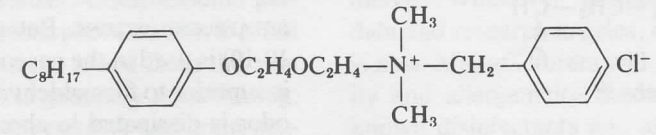
of anionic detergents reside in the negatively charged portion (anion) of the molecule. Anionic detergents are used in modern laundry powders. Like soaps, they mechanically remove microbial contaminants with the dirt and grime of soiled articles. Because of their surface tension—reducing qualities they are able to lyse some of the more delicate bacteria such as the pneumococci, and they are effective against a few of the enveloped viruses. They are not, however, considered disinfectants.

Cationic Detergents. Members of the cationic detergent group are among the most popular disinfectants, antiseptics, and sanitizers. Chemically they contain a positively charged "-onium" ion: the ammonium ion, phosphonium ion, sulfonium ion. Best known and most widely employed are the quaternary ammonium ion compounds, referred to in chemical slang as the "quats." A biologically active long aliphatic chain (at least 12 carbons in length) is attached to the ammonium ion as part of the structure of the cationic detergent. Cationic detergents thus contain a lipophilic group (the long alkyl chain) and a hydrophilic group (the charged portion of the molecule). The lipid-containing membrane of the bacterial cell attracts the lipophilic alkyl chain portion of the molecule. The charged hydrophilic portion of the molecule is oriented to the surrounding aqueous medium. The lethal action of the cationic detergents is primarily ascribed to cell membrane disruption, which, in turn, is due to orientation of the molecules at the lipid-water interface of the membrane. Enveloped viruses, because of their lipid content, are inactivated by cationic detergents. Protein denaturation and enzyme inactivation are believed to be other mechanisms of action of these detergents.

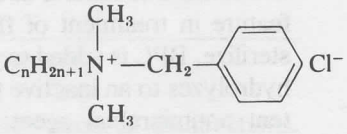
Hundreds of cationic detergents have been synthesized and tested, but only three main types are popularly used as antimicrobial agents: benzalkonium chloride (Zephiran), benzethonium chloride (Phemerol), and cetylpyridinium chloride (Ceepryn) (Fig. 10-10). The words in parentheses are the proprietary names of some of the better-known commercial products.

The popularity of the cationic detergents is due to their wide spectrum of activity against vegetative cells and enveloped viruses, and to the fact that they are bland (colorless, tasteless, odorless), very stable, nontoxic, inexpensive, and remain active at high dilutions. These good qualities have to be weighed against a number of shortcomings. They are not sporicidal or tuberculocidal. *Pseudo-*

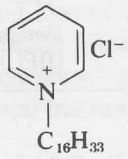
Fig. 10-10. Three popular cationic detergents.



Benzethonium chloride



Benzalkonium chloride



Cetylpyridinium chloride

monas organisms appear to be refractory to them. Cationic detergents are readily absorbed by surfaces such as gauze and cotton that are sometimes used to cushion instruments being decontaminated or being kept sterile in disinfectant solutions. Organic matter interferes with their action. They are readily neutralized by soaps and anionic detergents. If soap is used to remove gross contamination or soil from the skin or from an object, the follow-up use with a cationic detergent may be self-defeating and deceiving if a soap residue remains. The cationic detergent loses its antimicrobial activity because it chemically interacts with the soap.

Nonionic Detergents. The nonionic detergents have nonionized polar groups. They have no antimicrobial effect of themselves. Some serve as the organic carrier molecules of iodophors. Some are used as wetting agents in the laboratory to help break up clumps of adherent bacteria.

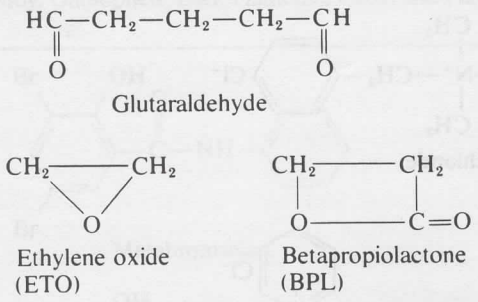
Alkylating Agents

A number of disinfectants exert their antimicrobial effects by substituting alkyl groups for the hydrogen of reactive groups of enzymes, nucleic acids, and proteins. Labile reactive hydrogens are available on carboxyl groups (–COOH), sulfhydryl groups (–SH), amino groups ($–NH_2$), and hydroxyl groups (–OH). Some of the alkylating agents are used in the gaseous or vapor phase, some as liquids, some in either the gaseous or the liquid state.

FORMALDEHYDE. Formaldehyde is sometimes employed in the gaseous state as a fumigant. In solution as formalin, it is known for its uses as a fixative for anatomical and pathological tissue specimens, as an inactivator in vaccines, and as a component of embalming fluids. Sterilization and disinfection applications are limited because it is a tissue irritant. It is poorly penetrating and slow acting, and it tends to polymerize, leaving a whitish deposit. It is sporicidal at high concentration. At one time it was used to sterilize catheters, endoscopes, and like instruments that were not easily sterilized by other means.

GLUTARALDEHYDE. A chemical relative of formaldehyde, glutaraldehyde has two aldehyde groups, one at either end of a five-carbon chain (1,5-pentanedial) (Fig. 10-11), and is therefore chemically categorized as a *dialdehyde*. It is one of the more recent additions to the disinfectant armamentarium and is highly rated as a disinfectant. It has a wide spectrum of activity. When used in a 2% solution, it is bactericidal, tuberculocidal, and virucidal in 10 minutes and sporicidal in 10 hours. Its action against the human hepatitis viruses has not been

Fig. 10-11. Alkylating agents.



thoroughly tested, but glutaraldehyde disinfectants along with iodine compounds and bleach are recommended for use against hepatitis viruses in situations in which sterilization is not feasible (e.g., countertops). There are both alkaline formulations and acid formulations. Since glutaraldehyde is irritating to the skin and mucous membranes and very irritating to the eyes, it is not used as an antiseptic. Commercially available glutaraldehyde preparations include Cidex, Sporicidin, Glutarex, and Sonacide.

ETHYLENE OXIDE. The gas ETO, a cyclic ether (Fig. 10-10), is gaining in popularity as a sterilizing agent as new means are devised to apply it. The major advantage of ETO is that this extremely penetrative gas is able to sterilize in the absence of high levels of heat or moisture. A wide range of articles that are affected by elevated heat are sterilized with ETO: catheters; disposable medical, dental, and laboratory items; dental handpieces; textiles; sutures; and heart-lung machines.

ETO forms explosive mixtures with air. ETO is, therefore, diluted with inert gases such as carbon dioxide, nitrogen, or halogenated hydrocarbons. ETO can be toxic if too much is inhaled, and it is a vesicant (i.e., it can cause blisters) on contact. It is slow acting, requiring 4 hours at 50–56°C or 6 to 12 hours at room temperature to produce sterility. Special equipment is necessary. Many hospitals now have ETO autoclaves as part of their standard sterilizing equipment. Items treated with ETO that are to be used in patient contact must first be aerated to remove the residual ETO.

BETAPROPIOLACTONE. Betapropiolactone (BPL) (Fig. 10-10) is used in the gaseous phase as a fumigant. It is superior to formaldehyde in that role because the odor is dissipated in about 48 hours. In the liquid phase it decomposes within a few hours to an inactive form. The natural decomposition is a desirable feature in treatment of fluids that are difficult to sterilize. BPL is added to the fluid, sterilizes it, and hydrolyzes to an inactive form. Despite being a potent antimicrobial agent, BPL has not achieved wide application. It is allegedly carcinogenic. The vapor is a vesicant and a lacrimator (increases the flow of tears).

Heavy Metals

Most of the heavy-metal disinfectants contain mercury or silver. Inorganic and organic preparations are available, but metal-containing disinfectants have been largely replaced by more effective compounds. They are slowly bactericidal, their action being principally bacteriostatic and reversible. Organomercurial antiseptics have lost the popularity they once held and have been superseded by iodine compounds, cationic detergent formulations, and alcohol. Mercury compounds are currently used as preservatives for biologicals. The one silver compound to be noted is silver nitrate. It is the law in most states that a 1% prophylactic silver nitrate solution be instilled into the eyes of all newborns to prevent the development of gonococcal conjunctivitis. A recent burn treatment technique also incorporates silver nitrate.

Other Classes of Disinfectants

Other chemical classes containing disinfectants are not as well known or as widely used as the compounds just discussed. They include dyes, nitrofurans, amidines, and essential oils.

Special mention is due an antiseptic with the generic name *chlorhexidine*. Chlorhexidine preparations have been employed abroad for many years. A 4% chlorhexidine preparation (the commercial product is called *Hibiclens*) was approved for use

in the United States in 1976. It is used for surgical scrubs, for hand washing by health care personnel, and as a skin wound cleanser. Chlorhexidine persists on the skin whereas iodophors do not; it is not appreciably absorbed into the blood from the skin as hexachlorophene is; it is generally nonirritating; and it is active against gram-positive and gram-negative bacteria.

SELECTION OF DISINFECTANTS

There are more than 8000 licensed disinfectants. No one disinfectant possesses all the qualities ideal for application to all microbial control problems.

Level of Disinfection

Different levels of disinfection are required in different situations. The use of disinfectants can be thought of in three broad application categories: (1) those used for housekeeping, sanitation, and environmental applications—on floors, furniture, and objects of the environment that may indirectly serve as a source of infectious disease; (2) those used for the disinfection of articles that come directly or indirectly into contact with the body: everything from instruments and thermometers to drawer pulls; and (3) those applied to the skin and mucous membranes. Sometimes the same chemical has a place in all three categories. Certain disinfectants are recommended for each category of application. The user should know several representative disinfectants in each category and have at least one of each type on hand (Table 10-4).

Trade or Proprietary Name

How is one to select an antiseptic or disinfectant on the basis of its trade or proprietary name? The chemical or generic name is usually printed on the container (Table 10-5). Basic knowledge of the action of the chemical can be obtained from a number of sources—for example, from textbooks in microbiology or pharmacology and from books specifically devoted to sterilization and disinfection. Some of the latter are cited under Additional Readings at the end of this chapter. Make purchases from reputable manufacturers or buy products that have been federally approved for interstate commerce. Whenever possible, examine published data and research articles, especially if the product is new. Manufacturers will supply reports on toxicity and allergenicity. Look for comparisons with known disinfectants and antiseptics. Examine the official publications of your professional organization. Dentistry, for example, has a biennial publication, *Accepted Dental Therapeutics*. Two of its chapters are entitled "Antiseptics and Germicides for Topical Application" and "Sterilization or Disinfection of Dental Instruments."

Practical Recommendations

To prevent infection of self, the cross infection of patients, and contamination of the environment, keep these suggestions in mind:

1. Allow disinfectants time to act.
2. Make liberal use of soap and water.
3. Keep immunizations current.
4. Keep contaminated fingers from the mouth and nose for they are the major portals of entry of microorganisms into the body.
5. Wear protective clothing whenever it is advised.
6. Most important, "think through" or "see in the mind's eye," as Lister phrased it, how microorganisms may be disseminated in an environment.

Imagine that the contaminating material is stained with a fluorescent, eye-catching dye. The contamination from a wound dressing might show up on the hands of the nurse, on the instruments that were used to remove the dressing, on the hospital bed stand, the bedclothes, the floor, the patient's gown, the nurse's clothing—anywhere in the room. Dental personnel might envision saliva as brilliantly colored. An instrument from the patient's mouth placed on a surface deposits saliva on that surface. An instrument to be used on another patient placed on that same spot becomes contaminated. With this mental picture should come the realization that every move must be made in such a manner as to provide the least number of opportunities for dis-

Table 10-4. Disinfectants and Antiseptics[a]

Class	Disinfectant[b]	Antiseptic	Other properties
GAS			
Ethylene oxide	+2 to +4	0	Toxic; good penetration; requires relative humidity of 30% or more; bactericidal activity varies with apparatus used; absorbed by porous materials. Dry spores highly resistant; moisture must be present, and presoaking is desirable.
LIQUID			
Glutaraldehyde, aqueous	+3	0	Sporicidal; active solution unstable; toxic.
Formaldehyde + alcohol	+2	0	Sporicidal; noxious fumes; toxic; volatile.
Formaldehyde, aqueous	+1 to +2	0	Sporicidal; noxious fumes; toxic.
Phenolic compounds	+3	±	Stable; corrosive; little inactivation by organic matter; irritate skin.
Chlorine compounds	+1	±	Flash action; much inactivation by organic matter; corrosive; irritate skin.
Alcohol	+2	+3	Rapidly -cidal; volatile; flammable; dries and irritates skin.
Iodine + alcohol	0	+4	Corrosive; very rapidly -cidal: causes staining; irritates skin; flammable.
Iodophors	+1	+3	Somewhat unstable; relatively bland; staining temporary; corrosive.
Iodine, aqueous	0	+2	Rapidly -cidal; corrosive; stains fabrics; stains and irritates skin.
Quaternary ammonium compounds	+1	+2	Bland; inactivated by soap and anionics; absorbed by fabrics.
Hexachlorophene	0	+2	Bland; insoluble in water, soluble in alcohol; not inactivated by soap; weakly -cidal.
Mercurial compounds	0	+1	Bland; much inactivated by organic matter; weakly -cidal.

± = some compounds may or may not be effective antiseptics.

[a]More detailed information must be obtained from descriptive brochures, journal articles, and books. Selection of the most appropriate germicide should be based on whether it is to be used as a disinfectant or as an antiseptic and on the estimated level of antimicrobial action needed.

[b]Maximal usefulness is denoted by +4.

Source: From E. H. Spaulding, *Manual of Clinical Microbiology* (2nd ed.). Washington, DC: American Society for Microbiology, 1974.

Table 10-5. Generic and Trade Names of Selected Antiseptics and Disinfectants

Generic or chemical name	Proprietary or trade name
PHENOLICS	
Amylphenol	Pentophen
Bithionol	Actamer, Lorothidol
Chlorophene	Santophen I
Chlorquinaldol	Steroxin
Dichlorophene	G-4, Didroxane
Diiodohydroxyquin	Floraquin, Yodoxin
Hexachlorophene	G-11, AT-7, Gamophen
Iodochlorhydroxyquin	Vioform, Nioform
o-Phenylphenol, orthoxenol	Dowicide 1
HALOGENS	
Halazone	Pantocid
Povidone-iodine	Isodine, Betadine, Prepodyne
Tetraiodopyrrole	Iodol
Thymol iodide	Aristol, Iodosol
Undecoylium chloride-iodine	Virac
CATIONIC DETERGENTS	
Benzalkonium chloride	Zephiran
Benzethonium chloride	Phemerol
Cetylpyridinium chloride	Ceepryn
Methylbenzethonium chloride	Diaparene
HEAVY-METAL COMPOUNDS—MERCURY	
Merbromin	Mercurochrome
Mercresol	Mercresin
Nitromersol	Metaphen
Thimerosal	Merthiolate
AMIDES, UREAS, AND CARBAMIC ACID DERIVATIVES	
Chlorhexidine	Hibitane
Cloflucarban	Irgasan CF3
Dibromsalan	Temasept II component
Tribromsalan	Tuasol 100
Triclocarban	TCC

seminating microorganisms. Asepsis procedures should not be performed in a perfunctory, indifferent manner even though it is virtually impossible to know that infection has been either prevented or caused because of one's application of, or failure to apply, the appropriate sterilization and disinfection measures.

SUMMARY

An understanding of the basic principles of sterilization and disinfection begins with an understanding of the commonly used terms of *sterile, -cide, -stasis, sepsis, asepsis, antisepsis, disinfection, sanitization*, and *germicide*. The composition of the population of microorganisms to be controlled usually is unknown, and therefore the assumption is made that highly resistant forms are present. Rarely is the level of kill in the population tested and therefore sterilization or disinfection procedures should be carried out as prescribed. There are variations in resistance levels within a population because of differences in metabolic states and in microenvironments. Certain microorganisms pose special problems, notably bacterial endospores because of their resistance to heat and disinfectants, mycobacteria because of their resistance to disinfectants, pseudomonad bacteria because of their ubiquity and resistance to antimicrobial agents, and the hepatitis viruses because of the mode of transmission. Interfering matter, especially that derived from the body (for example, blood), and environmental conditions affect the activity of antimicrobial procedures and agents. The principal modes of attaining sterility are autoclaving in saturated steam, elevated dry heat, exposure to ethylene oxide, and the Chemiclave, which provides exposure to the vapors of a combination of chemicals. Because sterilizing devices are subject to physical stresses and to contamination, periodic use of sterilization indicators is advised.

The physical methods of controlling microorganisms include the moist heat methods of boiling, saturated steam autoclaving, and pasteurization; the dry heat methods of elevated hot air temperatures and incineration; filtration of liquids and air, especially with membrane filters; ultrasonic cleaning for the purpose of removing interfering matter; irradiation with ultraviolet (germicidal) lamps for surface sterilization or disinfection; and irradiation of the ionizing type for the sterilization of disposable supplies.

There is no all-purpose disinfectant; the disinfec-

tant to be used must be chosen on the basis of its properties and the characteristics of the job at hand. The mode of action of chemical disinfectants may be to affect the lipid portion of the cell membrane, to oxidize or reduce an important functional group of an enzyme, or to coagulate microbial proteins. The evaluation of disinfectants involves initial testing (a first-performance or screening test) as well as in-use testing. Dilutions of pure phenol serve as the standard of comparison (the phenol coefficient test). The major classes of disinfectants are phenol and its derivatives, alcohols, halogen compounds, surfactants (soaps and detergents), alkylating agents, and heavy-metal compounds.

QUESTIONS FOR STUDY

1. Define *sepsis* and the derived terms *asepsis* and *antisepsis*.
2. Differentiate between disinfection and antisepsis.
3. Why are bacterial endospores, mycobacteria, hepatitis B virus, and pseudomonads regarded as difficult to control by chemical or physical means?
4. Why is the removal of pus, blood, or saliva so important when sterilizing or disinfecting?
5. Name the four major modes of attaining sterility and give at least one drawback of each mode.
6. How are microorganisms destroyed by moist heat?
7. What are the minimum conditions for attaining sterility when using saturated steam autoclaving?
8. Describe sterilization indicators. Include the advantages and disadvantages of each.
9. What is meant by the term *biological monitor*? Name the principal one.
10. Name two physical methods of attaining disinfection.
11. What articles typically are sterilized with elevated dry heat?
12. What are the minimum conditions for attaining sterility when using dry heat sterilization?
13. How are cold, filtration, and ultrasonics used in the control of microorganisms?
14. Cite two problems associated with the use of germicidal lamps.
15. Name the two major modes that are used to sterilize single-use disposable medical, dental, and laboratory supplies.
16. Cite five desirable qualities of an ideal disinfectant.
17. In general terms what are the modes of antimicrobial action of disinfectants?
18. What is the phenol coefficient test, and why is it important?
19. Why is phenol historically important? What are the good qualities of phenol compounds? What are the undesirable qualities?
20. Why is hexachlorophene now a prescription item?
21. What is the common name and the chemical name for a potent disinfectant that is a common household cleaning agent?
22. Distinguish between tincture of iodine and iodophors. Between soaps and detergents.
23. Cite the mode of action of cationic detergents and their good and bad qualities.
24. Cite the mode of action of the alkylating agents.
25. The necessity of aerating patient-contact arti-

cles after sterilization is a disadvantage of which mode of sterilization?

26. Mercresin, Merthiolate, and thimerosal are examples of what specific group of disinfectants?

27. The instillation of 1% silver nitrate into the eyes of newborns is done for what purpose?

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11. CHEMOTHERAPY

OBJECTIVES

To describe those factors that are important in determining the potential of a chemical as a chemotherapeutic agent

To list the major chemotherapeutic drugs that are inhibitors of cell wall synthesis, cytoplasmic membrane function, protein synthesis, nucleic acid synthesis, and cell metabolites

To briefly describe the mechanism of action of penicillins on the cell wall

To describe the various ways in which protein synthesis can be inhibited by chemotherapeutic agents

To describe the antimicrobial effect of drugs that are administered in combinations

To list the situations in which chemotherapeutic agents can be used for prophylaxis

To describe the methods by which microorganisms can demonstrate resistance to chemotherapeutic agents

To explain the methods for determining the antimicrobial activity of antibiotics in the clinical laboratory

To define: antibiotic, procaine, beta-lactamase, penicillin-binding proteins, imidazole, aminoglycoside, antimetabolite, Kirby-Bauer method

General Characteristics of Chemotherapeutic Agents
How to Determine Antimicrobial Activity
Factors Determining the Potential of a Chemotherapeutic Agent
- Solubility
- Stability
- Excretion Rate
- Permeability
- Hypersensitivity
- Activity Spectrum
- Drug Resistance
- Toxicity

Antimicrobial Drugs
- Inhibitors of Cell Wall Synthesis
  - Penicillin
    - Mechanism of Action
  - Cephalosporins
  - Bacitracin
  - Vancomycin
- Inhibitors of Cytoplasmic Membrane Function
  - Polyenes
  - Imidazoles
  - Polymyxins
- Inhibitors of Protein Synthesis
  - Aminoglycosides
  - Tetracyclines
  - Chloramphenicol
  - Erythromycin
  - Lincomycin and Clindamycin
  - Spectinomycin
- Inhibitors of Nucleic Acid Synthesis
  - Rifampin
  - Nalidixic Acid
  - Flucytosine
- Antimetabolites
  - Sulfonamides
  - Para-aminosalicylic Acid and the Sulfones
  - Trimethoprim
  - Ethambutol
  - Isoniazid
  - Nitrofurans

Factors in Selecting a Drug for Chemotherapy
- Dosage
- Route of Administration
- Drug Combinations
- Prophylaxis
- Toxicity

Drug Resistance
- Interference with Transport
- Enzymatic Inactivation
- Alteration of the Microbial Target
- Synthesis of Resistant Metabolic Pathways

Quantitative Determination of Antibiotic Activity
- Broth Dilution Test
- Agar Dilution Method
- Disk Diffusion Test
- Antibiotic Concentrations in the Patient's Serum

Chemotherapeutic agents are a class of compounds used in the treatment of disease. They fall into two general categories: those produced synthetically by chemists and those produced biologically by microorganisms, that is, *antibiotics*. This separation is rather imprecise because, since the discovery of antibiotics over 50 years ago, scientists can produce semisynthetic chemotherapeutic agents. To produce semisynthetic compounds, the nucleus of a biologically synthesized compound is recovered from the producing organism and various chemical groups are synthetically added to it.

Chemotherapeutic agents are not new to our civilization. Although their widespread application in the treatment of disease is of modern origin, the recorded use of these agents began in the early seventeenth century. During that period plant extracts such as quinine were used in the treatment of malaria and amebic dysentery. Only in the late nineteenth and early twentieth centuries did humans begin to synthesize compounds capable of acting as chemotherapeutic agents. Since 1930 scientists have isolated, from certain strains of bacteria and molds, compounds with various degrees of antimicrobial activity. These microorganisms, which are found in the soil, are capable of producing only modest amounts of antimicrobials. Microbiologists seized on this natural mechanism and in the labo-

Table 11-1. Characteristics of Some Important Chemotherapeutic Agents

Drug	Action		Activity Spectrum		Mechanism of action
	-static	-cidal	Gram-positive	Gram-negative	
Aminoglycosides (amikacin, neomycin, streptomycin, gentamicin, kanamycin, etc.)		X	X	X	Inhibit protein synthesis
Amphotericin B	Fungistatic		Fungi		Cell membrane inhibitor
Bacitracin		X	X		Cell wall inhibitor
Cephalosporins (cephalothin, cefoxitin, etc.)		X	X	X	Cell wall inhibitors
Chloramphenicol	X		X	X	Inhibits protein synthesis
Clindamycin	X		X		Inhibits protein synthesis
Erythromycin	X		X		Inhibits protein synthesis
Flucytosine	Fungistatic		Fungi		Inhibits protein synthesis
Isoniazid	X	X	*Mycobacterium tuberculosis*		Competes with NAD
Lincomycin	X		X		Inhibits protein synthesis
Nalidixic acid	X			X	Inhibits DNA synthesis
Nitrofurantoin		X	X	X	?
Para-aminosalicylic acid	X		*Mycobacterium tuberculosis*		Antimetabolite
Penicillins		X			
Penicillin G		X	X		Cell wall inhibitor
Ampicillin		X	X	X	Cell wall inhibitor
Carbenicillin		X	X	X	Cell wall inhibitor
Polymyxins		X		X	Cell membrane inhibitors
Rifampin	X		X		Inhibits RNA synthesis
Sulfonamides (sulfanilamide, etc.)	X		X	X	Antimetabolites
Tetracyclines	X		X	X	Inhibit protein synthesis
Vancomycin		X	X		Cell wall inhibitor

NAD = nicotinamide-adenine dinucleotide.

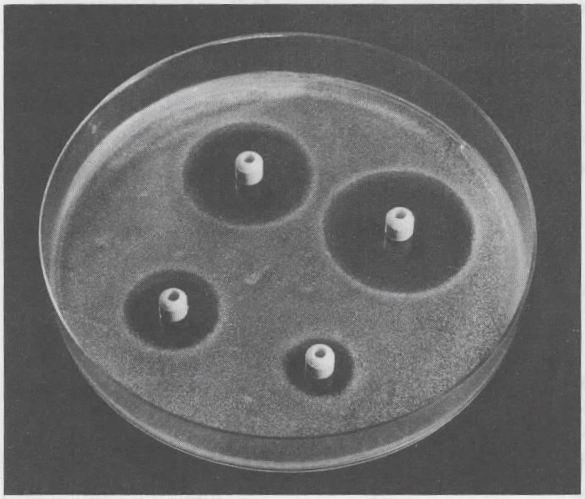
ratory manipulated the microorganisms so that large amounts of antibiotics could be produced. Although only a small percentage of these drugs have been suitable for treating human subjects, practically every major microbial (but not viral) disease has been subject to some control by them. Table 11-1 reviews the properties of the major chemotherapeutic agents used in the treatment of infectious disease.

One of the purposes of this chapter is to describe the characteristics of the various antibiotics and synthetic chemotherapeutic agents. Second, we wish to describe the rationale for the selection and use of chemotherapeutic agents in the clinical situation.

GENERAL CHARACTERISTICS OF CHEMOTHERAPEUTIC AGENTS

A chemotherapeutic agent is one that is selectively toxic, that is, it is toxic to the microbial cell but not to the host cell. Selective action is possible because of differences in the biochemistry of the host and the microbial cell. For example, penicillin inhibits the synthesis of bacterial cell wall, a component

Fig. 11-1. Cylinder method for determining bacterial sensitivity to drugs. Cylinders are placed on agar plates and filled with a specified quantity of antibiotic. Antibiotic is considered effective if a zone of inhibition develops. Note the variation in the width of zones of inhibition. The width may be due to the type of antibiotic placed in the cylinders or to a variation in the concentration of one antibiotic. (From L. P. Garrod and F. O'Grady, *Antibiotic and Chemotherapy* [5th ed.]. London: Churchill Livingstone, 1973.)



that is not present in mammalian cells. In addition, even though some biochemical activities are similar in the two types of cells, a chemotherapeutic agent has a greater affinity for the bacterial component. The selective toxicity of some drugs is so low that the therapeutic concentration of drug is very close to the concentration that is toxic for tissue. The ratio of therapeutic to toxic concentration is called the *therapeutic index* of the drug.

Chemotherapeutic agents have been classified according to their spectrum of activity—that is, which groups of microorganisms they inhibit. A broad-spectrum drug is one that has activity against gram-positive as well as gram-negative species. A narrow-spectrum drug affects only one group of microorganisms or perhaps only one species.

HOW TO DETERMINE ANTIMICROBIAL ACTIVITY

The antibiotic activity of a microbe isolated from the soil can be measured in a number of ways. In the plate diffusion methods, agar blocks containing a culture of the microorganism producing the antibiotic are placed on an agar surface seeded with a test microorganism. Antibiotic activity is noted as a clear zone around the agar block. A second test involves use of a culture filtrate from an antibiotic-producing microorganism or a synthetic agent made in the laboratory. The potential chemotherapeutic agent can be put into regularly spaced holes punched into the agar, or it may be introduced into cylinders placed on the surface of an agar plate seeded with the test microorganism. Antibacterial activity is again noted as a clear zone around the agar well or the cylinder (Fig. 11-1).

Once the potential chemotherapeutic agent has been shown to possess antibacterial activity, it must be analyzed for various biological properties such as spectrum of activity and cross resistance with other drugs. It must also be purified and analyzed chemically to determine whether it is really a new therapeutic agent and not one that has already been discovered.

FACTORS DETERMINING THE POTENTIAL OF A CHEMOTHERAPEUTIC AGENT

Any synthetic or naturally produced chemical substance must meet two important criteria before it can be considered for use as a chemotherapeutic agent: It must exert antimicrobial activity at high dilution when introduced into the body of the infected host, and it should be relatively nontoxic to the host. Other factors that play a role in determining the potential of a chemotherapeutic agent are included under the following topics.

SOLUBILITY

The purified drug should be soluble in water or some other innocuous compound and relatively

stable in solution. Few drugs on the market today are not soluble in water. Lipid solubility may be important if the drug is to be used for infections of the central nervous system. Lipid-soluble drugs can penetrate the membrane barrier of nervous tissue more easily than can lipid-insoluble ones.

STABILITY

Many drugs do not have a long shelf life and are inactivated at normal refrigeration temperatures. Administration of outdated antibiotics has been known to cause serious illness. The tetracyclines, for example, have been implicated in such situations.

EXCRETION RATE

Some drugs are rapidly excreted by the kidneys. The lower the excretion rate, the smaller the dose of antibiotic required to maintain high concentrations of the drug in infected tissue.

PERMEABILITY

Drugs vary in their ability to be absorbed from different tissues such as the gut and bloodstream. Most drugs show poor permeability into nervous tissue. For this reason, infections of the nervous system, such as meningitis, are often difficult to treat.

HYPERSENSITIVITY

Some individuals are allergic to drugs. Allergic reaction is particularly likely when the antibiotic used in treatment is penicillin or its derivatives. This sensitivity (5 to 8 percent of the population) is believed to be due to a product of penicillin degradation that reacts with a body protein, the combination of which acts as a foreign antigen. Other drugs that are known to elicit a hypersensitivity reaction include the sulfonamides, nalidixic acid, and streptomycin.

ACTIVITY SPECTRUM

The greater the number of gram-positive and gram-negative microorganisms affected by the antibiotic, the greater its applicability in treating disease. Some drugs, however, are effective against only a few species of microorganisms. This selectivity may be important if these species are resistant to most other antibiotics.

DRUG RESISTANCE

Pathogens show varying degrees of drug resistance. When choosing an antibiotic for therapeutic use one must be sure that the rate at which drug-resistant forms develop is low. The indiscriminate use of most drugs increases the probability of the patient being infected by drug-resistant forms.

TOXICITY

Any drug that is to be used in or on the body must be selectively toxic—that is, toxic to the microbe and not the host. Except for penicillin, most chemotherapeutic agents are somewhat toxic and interfere with host metabolism. The interference is usually not enough to hinder normal bodily functions.

ANTIMICROBIAL DRUGS

The effectiveness of chemotherapeutic agents is often related to their ability to arrest the growth of the infectious agent. Thus the host is able to wield his or her own defensive arsenal: phagocytosis and antibody production. If antibodies can be stimulated, future infections from the same infectious agent may in many instances be handled by the host without the intervention of antimicrobials. This is the principle of the vaccination process. In general, the antimicrobial activity of chemothera-

peutic agents relates to their ability to affect metabolism in several ways.

INHIBITORS OF CELL WALL SYNTHESIS

The most important drugs that inhibit cell wall synthesis are the penicillins, the cephalosporins, bacitracin, and vancomycin. These drugs are effective only on bacterial cells that are actively synthesizing cell wall peptidoglycan precursors.

Penicillin

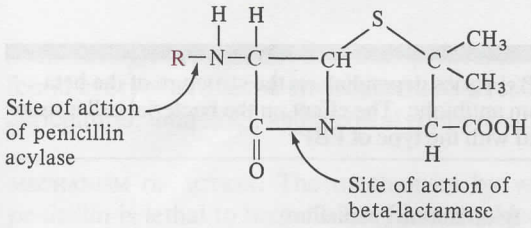
Penicillin comes as close as any drug to being the model antibiotic because of its low toxicity and the minimal concentration required for antibacterial activity. All the penicillins have a common nucleus and differ only in their laboratory-synthesized side chains (Fig. 11-2). Except for penicillin G (benzyl penicillin), all the penicillins are semisynthetic. The laboratory synthesis of the side chains has resulted in three classes of penicillins important for the following properties:

1. Acid resistance. Penicillin G, the only natural penicillin produced by the mold *Penicillium*, is relatively unstable to acid. Thus the drug would not be effective if given orally because of acid inactivation in the intestinal tract. Development of phenoxymethyl and phenoxyethyl penicillins has circumvented this undesirable property.
2. Penicillinase resistance. Many microorganisms, especially the pathogenic staphylococci, carry genes that code for an enzyme capable of inactivating penicillin. This enzyme is called *penicillinase* and is a type of *beta-lactamase* (because of its ability to cleave the beta-lactam ring of penicillin (Fig. 11-2). The penicillinase-resistant penicillins are methicillin, oxacillin, cloxacillin, dicloxacillin, and nafcillin. All except methicillin are also acid resistant.
3. Broad spectrum. Most penicillins are effective only against gram-positive microorganisms. This antimicrobial spectrum has been extended to include gram-negative microorganisms through the development of ampicillin, carbenicillin, and ticarcillin. Modifications of these drugs has resulted in compounds that produce higher serum levels after oral administration and have a broader spectrum of activity against gram-negative bacteria. These drugs include azlocillin, mezlocillin, piperacillin, amoxicillin, and amdinocillin. The effectiveness of these drugs is associated with their affinity for penicillin-binding proteins (see Mechanism of Action).

Special dosage forms of penicillin are often used in treatment. The potassium salt of penicillin G is dissolved very rapidly in body fluids. Even with intramuscular injections penicillin is quickly absorbed from the site of injection into the bloodstream. The rate of dissolution of penicillin can be reduced if the penicillin is combined with *procaine*. A single intramuscular injection of procaine-penicillin can produce a therapeutic level of drug for 24 hours in the bloodstream, whereas the same dose of penicillin in water is effective for only 4–5 hours. In its aqueous form penicillin is excreted rapidly from the kidneys. Probenecid is an organic acid that lowers the rate of excretion by competing with penicillin for the renal tubular secretory process. When probenecid and penicillin are administered together, excretion of penicillin from the body is greatly reduced. Probenecid is used infrequently today and is being supplanted by the preparation *benzathine penicillin*. The latter consists of two molecules of benzyl penicillin bound to dibenzylethylenediamine. Given intramuscularly, this preparation produces high levels of the drug in the serum for several days.

Penicillin and its derivatives are the least toxic of all drugs used in chemotherapy. When doses approach 40 to 50 million units, however, neurotoxicity can result. The most adverse effect caused by administration of penicillin is an allergic response, observed in up to 8 percent of the population who receive the drug. All persons are at potential risk of

Fig. 11-2. Structure of the penicillin nucleus (6-aminopenicillanic acid) and some of its more clinically useful derivatives.



Penicillin nucleus

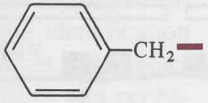
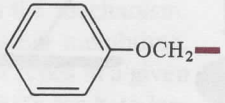
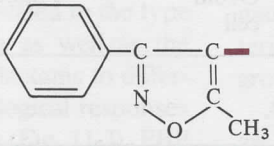
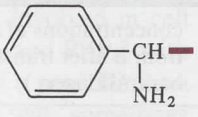
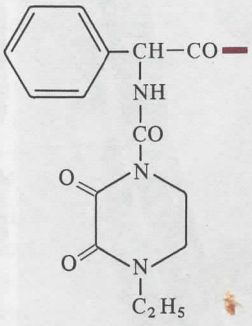
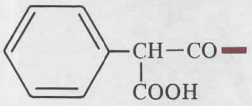
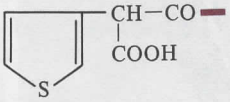
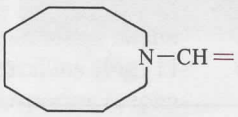
NAME OF DERIVATIVE	SIDE CHAIN	CHARACTERISTICS
Penicillin G (benzyl penicillin)		Inexpensive, acid labile, susceptible to beta-lactamases of gram-negative and gram-positive bacteria; ineffective orally
Penicillin V (phenoxymethyl penicillin)		Acid resistant; can be taken orally; narrow spectrum
Oxacillin		Can be taken orally; not susceptible to beta-lactamases; narrow spectrum
Ampicillin		Sensitive to beta-lactamases; broad spectrum; acid resistant
Piperacillin		Similar to ampicillin in activity; inhibits *Klebsiella;* resistant to carbenicillin; inhibits *Pseudomonas;* more resistant to cephalosporinases than other beta-lactam antibiotics
Carbenicillin		Cannot be administered orally; resistant to gram-negative beta-lactamases; active against *Pseudomonas*
Ticarcillin		Similar to carbenicillin
Amdinocillin (mecillinam)		Not effective against *Staphylococcus* and some gram-negative bacteria but effective against *E. coli, Klebsiella,* and some enterobacteriaceae resistant to ampicillin

Fig. 11-3. Movement of beta-lactams through the cell envelope and their effect on the bacterial cell. The affinity of a beta-lactam for penicillin-binding proteins (*PBP's*) varies depending on the structure of the beta-lactam antibiotic. The effect on the bacterial cell is associated with the type of PBP.

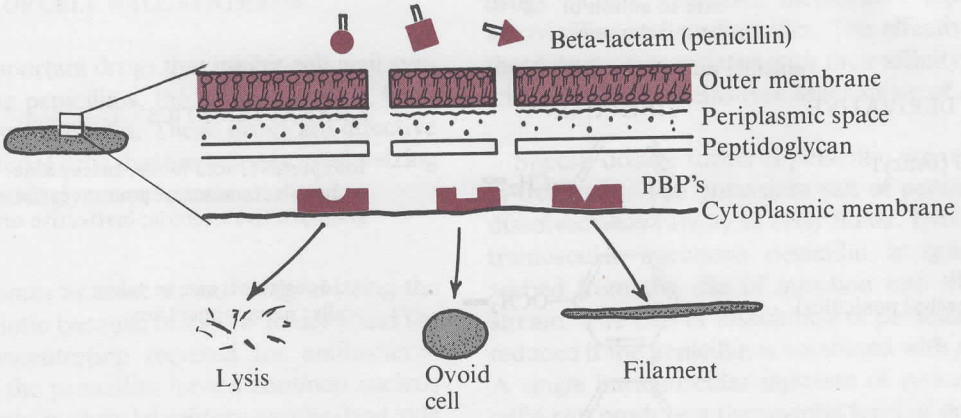
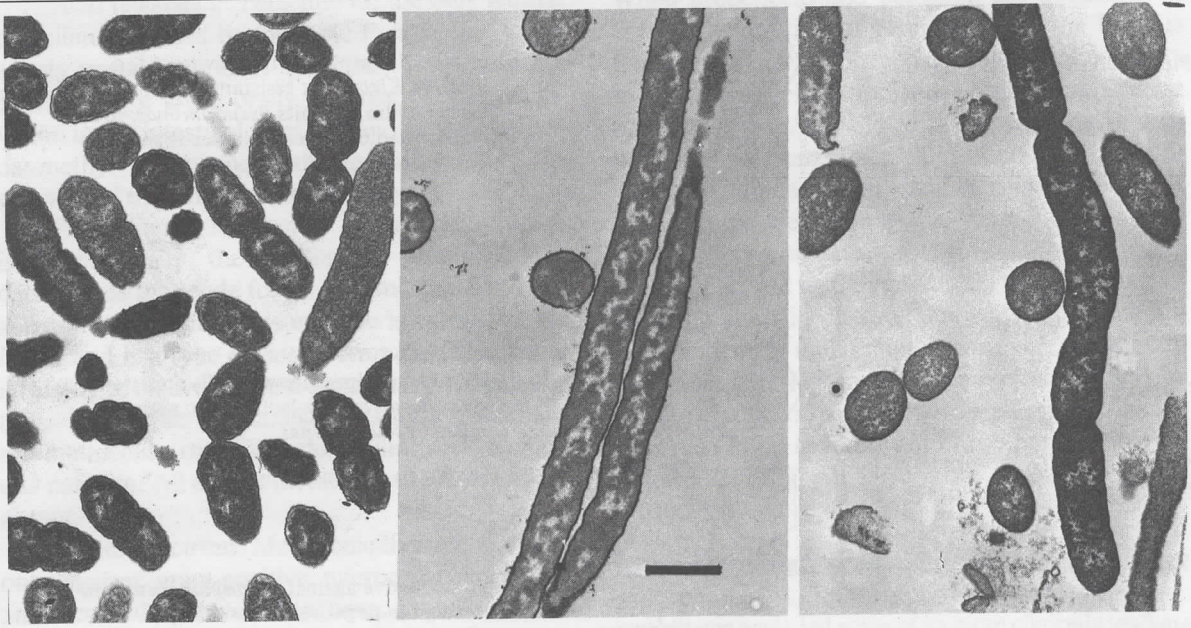


Fig. 11-4. Effect of subinhibitory concentrations of ampicillin on the gram-negative bacillus *Proteus mirabilis*. A. Control. B. *P. mirabilis* exposed to subinhibitory concentrations of ampicillin. C. *P. mirabilis* filaments from B after transfer to drug-free agar. (Courtesy Barbara Atkinson.)



A B C

such allergic reactions because penicillins are found everywhere in the environment as a result of agricultural, industrial, and medical contamination.

MECHANISM OF ACTION. The mechanism by which penicillin is lethal to bacteria has been a subject of controversy for many years. The exact mechanism is still not known, and more than one mechanism may actually be involved. The discovery of *penicillin-binding proteins* (PBP's) and the identification of a transpeptidase as the lethal target of beta-lactam antibiotics, such as the penicillins and cephalosporins, has shed some light on the mechanism. PBP's are located in the cytoplasmic membrane, and there are three to eight distinct types in a given organism. The affinity of these PBP's for beta-lactam antibiotics is variable and is related to the type and concentration of beta-lactam as well as the type of PBP. The binding of beta-lactams to different PBP's affects certain morphological responses associated with cell wall synthesis (Fig. 11-3). PBP I is associated with the enzymes involved in cell elongation, PBP 2 with cell shape, and PBP 3 with septum formation. The addition of a penicillin to a culture of rod-shaped bacteria will sometimes cause immediate lysis, while with other penicillins the cells may round up, forming ovoid cells, and with yet other penicillins there may be the formation of filaments (Fig. 11-4). It now appears that the initial action of beta-lactams is to inhibit bacterial growth by affecting the activity of an enzyme involved in peptidoglycan metabolism. The most likely candidate for this is a transpeptidase, that is, the enzyme that links the peptides in the peptidoglycan strands. This initial response is believed to be followed by an irreversible response in which one of the hydrolytic enzymes acts autocatalytically and breaks the covalent bonds in the peptidoglycan strands, thus exposing the cytoplasmic membrane to the environment. This ultimately leads to lysis of the cell (see Fig. 11-3).

Cephalosporins

Cephalosporins are beta-lactam antibiotics similar in structure and activity to the penicillins (Fig. 11-5). The naturally occurring cephalosporins (cephalosporin C) were first isolated from a fungus in 1945, and all had minimal antimicrobial activity. Semisynthetic derivatives have since been prepared with cephalothin the first marketed derivative of cephalosporin C. One of the most important properties of many of the cephalosporins is their resistance to the beta-lactamases of gram-positive bacteria that inactivate the penicillins. Cephalosporins are useful alternatives to the penicillins, particularly in the treatment of disease caused by gram-positive species resistant to penicillin and in situations in which the patient is allergic to penicillin. There are now second- and third-generation cephalosporins (Fig. 11-5), including cefamandole, cefazolin, cefoxitin, cefotaxime, and cefoperazone. Some of these new cephalosporins such as cefamandole have activity against gram-negative bacteria and are resistant to the beta-lactamases of this group of bacteria.

A group of cephalosporins called *cephamycins* are also unusually resistant to beta-lactamase of gram-negative bacteria. A semisynthetic cephamycin called *cefoxitin* has a broad spectrum of activity that includes gram-negative anaerobes such as *Bacteroides fragilis*.

A new class of beta-lactams that is structurally related to the cephalosporins is currently under investigation. The first of these to be evaluated clinically is called *moxalactam* (Fig. 11-6). It appears to have greater antibacterial activity than the conventional cephalosporins.

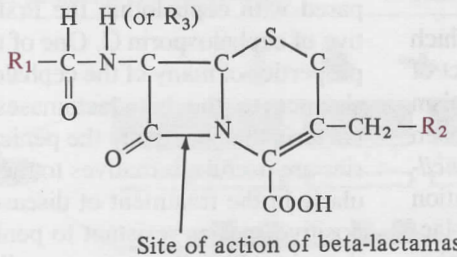
The mechanism of action of the cephalosporins is similar to that of the penicillins since it is the beta-lactam ring that exhibits specificity for the penicillin-binding proteins.

Bacitracin

Bacitracin (Fig. 11-7) is a peptide antibiotic originally isolated from the bacterium *Bacillus subtilis*. It is active against gram-positive bacteria but not gram-negative species. The drug is not absorbed from the intestinal tract and is very toxic to the kidneys (nephrotoxicity). Its primary use has been as a topical agent in combination with other topical agents such as polymyxin, neomycin, or both.

Bacitracin inhibits cell wall synthesis by prevent-

Fig. 11-5. Structure of the cephalosporin nucleus (7-aminocephalosporanic acid) and some of its more important derivatives. (IM = intramuscularly; IV = intravenously.)



Site of action of beta-lactamase (cephalosporinase)

Cephalosporin nucleus

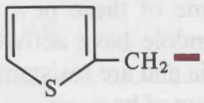
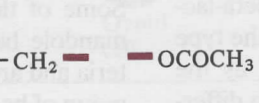
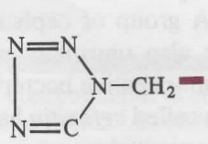
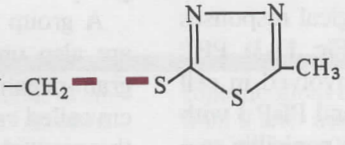
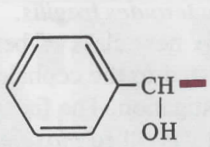
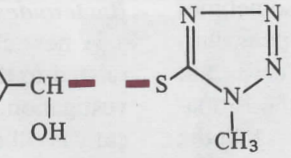
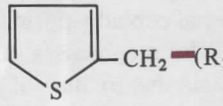
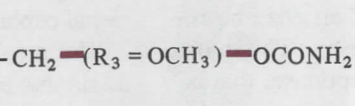
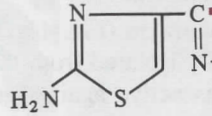
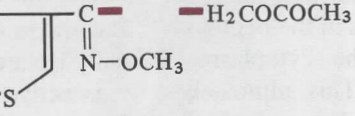
NAME OF DERIVATIVE	SIDE CHAIN R_1	SIDE CHAIN R_2	CHARACTERISTICS
Cephalothin			Can be given only IM or IV; used for severe staphylococcal infections
Cefazolin			Can be given only IM or IV; more active than cephalothin against *E. coli* and *Klebsiella;* more susceptible to beta-lactamase than cephalothin
Cefamandole			Inhibits enterobacteriaceae to greater degree than cephalothin; active against *Hemophilus influenzae;* administered parenterally
Cefoxitin			Diminished activity against gram-positives but greater activity against gram-negative organisms than cephamanadole; administered primarily IV
Cefotaxime			Marked resistance to beta-lactamases of certain gram-negative bacteria; active against *Pseudomonas*

Fig. 11-6. Structure of moxalactam, a new beta-lactam drug.

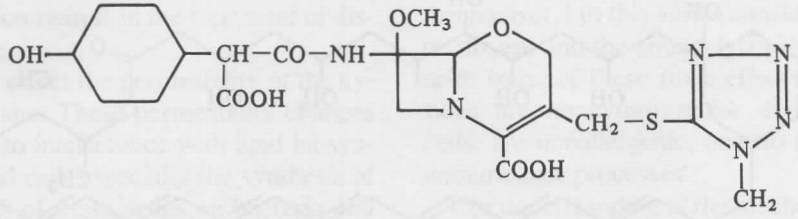
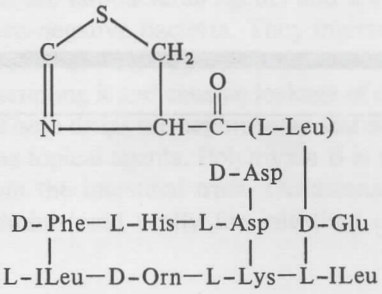


Fig. 11-7. Structure of bacitracin. (Leu = leucine; Glu = glutamic acid; Ileu = isoleucine; Lys = lysine; Orn = ornithine; Phe = phenylalanine; His = histidine; Asp = aspartic acid.)



ing the transfer of peptidoglycan precursors to the growing cell wall.

Vancomycin

Vancomycin is a drug with limited usefulness in the clinical situation. It is moderately toxic to the ear (ototoxicity) and kidneys. Its use has been restricted to the treatment of staphylococcal infections in which the infectious agent is resistant to methicillin or oxacillin. Almost all strains of *Staphylococcus aureus* resistant to these penicillins are sensitive to vancomycin. Vancomycin is also used in the treatment of antibiotic-associated pseudomembranous colitis (see Chapter 21).

The exact site of action of vancomycin during cell wall synthesis is not known, but it does block the synthesis of peptidoglycan with the accumulation of cell wall intermediates. Vancomycin is also believed to alter the permeability of the cytoplasmic membrane.

INHIBITORS OF CYTOPLASMIC MEMBRANE FUNCTION

Polyenes

Polyenes are antibiotics that are used primarily as antifungal agents and are produced mainly by Streptomycetaceae. The principal polyenes are amphotericin B (Fig. 11-8) and nystatin, but minor ones include candicidin, pimaricin, trichomycin, and hamycin. The mechanism of action of these drugs is similar. They are all lipophilic and bind irreversibly to cytoplasmic membrane sterols such as ergosterol. Membrane integrity is lost when polyenes bind, and this results in permeability changes. There is leakage of potassium ion, sugar, and other compounds plus impaired uptake of nutrients such as amino acids. The membranes surrounding the various fungal organelles are similarly affected by these antibiotics.

Amphotericin B, like other polyenes, has a low solubility in water, and most preparations are made up in sodium deoxycholate. The polyenes are relatively toxic drugs, and considerable care must be taken in their administration. Except for amphotericin B the polyenes are seldom administrated parenterally. Amphotericin B is used in the treatment of serious systemic fungal diseases such as histoplasmosis and blastomycosis. The concentration of

Fig. 11-8. Structure of amphotericin B.

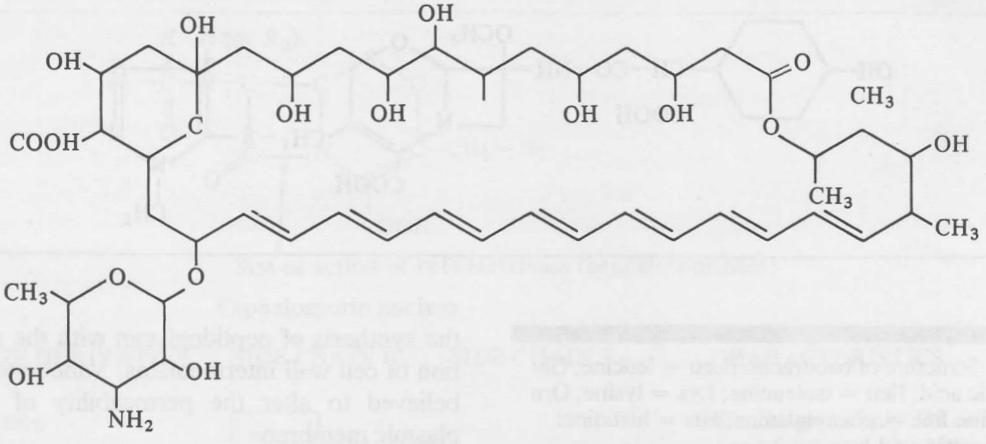
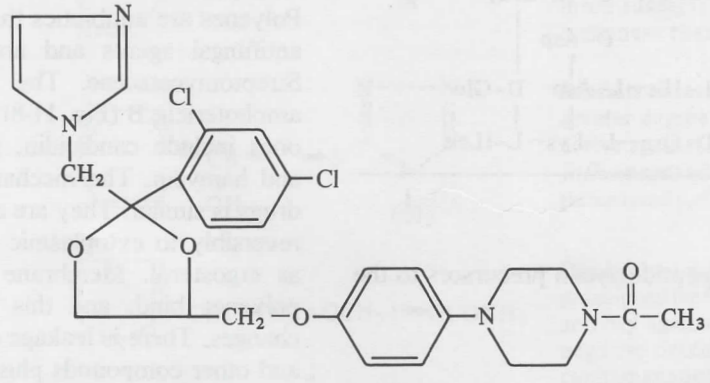


Fig. 11-9. Structure of one of the newest imidazoles, ketoconazole.



drug that is effective in treatment is very close to the dosage that is also very toxic. Amphotericin B is often administered with flucytosine—a combination that permits the use of smaller doses of amphotericin B.

Nystatin, the second most widely used polyene, is used primarily as a topical agent in the treatment of disease caused by the yeast *Candida*.

Imidazoles

The imidazoles are primarily antifungal agents but some show antibacterial activity. They have a broad spectrum of fungal activity and are active against yeasts and molds. The major imidazoles are miconazole, clotrimazole, and econazole. They are used primarily as topical agents because it is difficult to maintain effective levels of the drug in the bloodstream for sufficient periods of time.

One of the newest imidazoles is ketoconazole (Fig. 11-9). This drug has a broad spectrum of ac-

tivity and can be taken orally. It is well tolerated by patients and can be administered over long periods of time. These attributes make it an important alternative to amphotericin B in the treatment of disseminated disease.

The imidazoles affect the permeability of the cytoplasmic membrane. These permeability changes appear to be due to interference with lipid biosynthesis in the fungal cell, especially the synthesis of sterols. The effect of these drugs on bacteria and protozoa suggests that imidazoles may also influence the biosynthesis of lipid triglycerides.

Polymyxins

The polymyxins are polypeptide antibiotics produced by *Bacillus polymyxa*. Only polymyxin B and polymyxin E (colistin) are used clinically. The polymyxins are antibacterial agents and are active against gram-negative bacteria. They interact with phospholipids in the cytoplasmic membrane, thereby disrupting it and causing leakage of cellular contents. These drugs are nephrotoxic and are used primarily as topical agents. Polymyxin B is not absorbed from the intestinal tract. Occasionally the drug is administered orally for intestinal chemotherapy.

INHIBITORS OF PROTEIN SYNTHESIS

Aminoglycosides

Some of the most important antibiotics used in the treatment of bacterial diseases are those classified as inhibitors of protein synthesis. The major chemically related group of inhibitors is called the *aminoglycosides*. They consist of an amino ring structure to which are attached amino and nonamino sugars. The aminoglycosides are bactericidal against a wide variety of bacteria including staphylococci and the facultative and aerobic gram-negative bacilli. Some of the important aminoglycosides are streptomycin, amikacin, gentamicin, kanamycin, tobramycin, and others, which will be shortly discussed. The aminoglycosides are uniformly nephrotoxic and bring about morphological as well as functional alterations of the proximal renal tubules. With rational administration nephrotoxicity is minimal and is usually reversible. The aminoglycosides are also toxic to the hearing mechanism, but this varies considerably depending on dosage and the aminoglycoside being used. Despite some of these toxic effects the aminoglycosides are noncytotoxic for dividing mammalian cells, are nonallergenic, and do not interfere with immunologic processes.

Our understanding of the mechanism of action of the aminoglycosides is based on studies made with streptomycin. The other aminoglycosides are believed to act in a similar fashion. Exposure of the bacterial cell to streptomycin results first in a loss of potassium from the cell, followed by inhibition of protein, DNA, and RNA synthesis. The primary target of the drug is the bacterial ribosome. Streptomycin binds to a protein on the 30S ribosome, causing a distortion in the acceptor (A) site. This distortion affects the binding of aminoacyl transfer RNA (tRNA) and peptidyl tRNA and eventually results in the inhibition of polypeptide chain elongation. Streptomycin also increases the frequency of misreading of the genetic code owing to incorrect codon-anticodon interaction. Therefore premature nonsense codons are occasionally formed and incomplete polypeptides are synthesized. The lethal effects of streptomycin, however, are due to the irreversible binding of the drug to the 30S ribosome and the cell's inability to manufacture essential proteins.

A variety of new naturally occurring and some semisynthetic aminoglycosides have been discovered and developed, respectively, in the past few years. The activities of these new aminoglycosides as well as the characteristics of the older drugs are outlined in Table 11-2.

Tetracyclines

Tetracyclines (Fig. 11-10) are bacteriostatic agents that inhibit protein synthesis by binding to the 30S ribosome and blocking the binding of aminoacyl tRNA to the A site. Tetracyclines have the broadest spectrum of activity of all antibiotics. They inhibit gram-positive and gram-negative bacteria, the rickettsiae, mycoplasmas, spirochetes, chlamyd-

Table 11-2. Characteristics of the Aminoglycosides

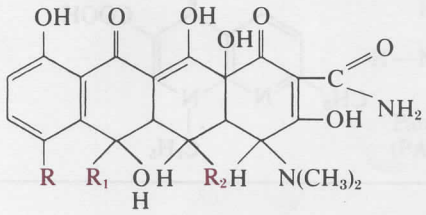
Aminoglycoside	Principal clinical use	Comments
Streptomycin	Treatment of tuberculosis but always with other drugs; treatment of tularemia and plague; combined with penicillin in treatment of endocarditis caused by enterococci and viridans streptococci	Most microorganisms develop resistance to drug when used alone; therefore it is used only under special circumstances
Kanamycin	Used primarily as a topical drug or for oral therapy	Lacks activity against pseudomonads; susceptible to inactivation by a number of aminoglycoside-modifying enzymes
Gentamicin	Widely used in treatment of infections caused by facultative and aerobic bacilli, for example, the Enterobacteriaceae	Effective against pseudomonads and most Enterobacteriaceae; resistant strains found primarily in hospitals
Tobramycin	Spectrum of activity similar to gentamicin's	More active against *Pseudomonas aeruginosa* than is gentamicin; resistant strains primarily in hospitals
Sisomicin	Spectrum of activity similar to gentamicin's	Similar to tobramycin in its activity against *P. aeruginosa*
Amikacin	Has broadest spectrum of activity of aminoglycosides; particularly effective against *Pseudomonas* species	Produced from the acylation of kanamycin; resistant to aminoglycoside-modifying enzymes; no major resistant strains detected
Neomycin	Used primarily as a topical agent because of toxicity; activity spectrum similar to kanamycin's	Occasionally administered orally as an antiseptic before bowel surgery
Fortimicin A	Activity parallels that of amikacin but has greater activity against *Serratia marcescens*	Resembles kanamycin in its poor activity against *P. aeruginosa*
Netilmicin	Resistant to enzymes that modify gentamicin and sisomicin; effective against more strains of enteric bacilli than is gentamicin	A semisynthetic derivative of sisomicin
5-Episisomicin	More active against *P. aeruginosa, S. marcescens, Providencia* species than is gentamicin	A semisynthetic derivative of sisomicin

iae, and some protozoa. Although tetracyclines are relatively nontoxic, there are situations in which their use is not appropriate. Continued use of tetracyclines eliminates the bacterial flora and predisposes the patient to disease by indigenous fungi, such as species of *Candida*, a condition called superinfection. Tetracyclines stain developing teeth, and the drug should not be administered to children under 5 years of age. Tetracyclines also cause fatty degeneration of the liver in pregnant women; other drugs should be considered to treat infections of this group. Tetracyclines are the drug of choice for infections caused by rickettsiae and chlamydiae and are frequently used in the treatment of upper respiratory diseases. Doxycycline and minocycline are absorbed more efficiently than are other tetracyclines. Minocycline is active against some bacteria resistant to other tetracyclines.

Chloramphenicol

Chloramphenicol is a bacteriostatic agent (Fig. 11-11) that inhibits protein synthesis by binding to the 50S ribosome and blocking peptidyl transfer. It has a broad activity spectrum similar to that of the tetracyclines but is more toxic and is used only under special circumstances. Toxicity is associated

Fig. 11-10. Structure of the tetracycline nucleus and some of its derivatives.

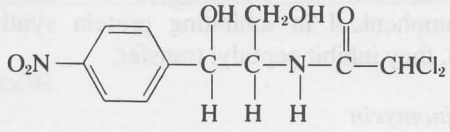


Tetracycline nucleus

	R	R_1	R_2
Chlortetracycline	—Cl	$-CH_3$	—H
Oxytetracycline	—H	$-CH_3$	—OH
Tetracycline	—H	$-CH_3$	—H
Demeclocycline	—Cl	—H	—H
Methacycline	—H	$=CH_2$*	—OH
Doxycycline	—H	$-CH_3$*	—OH
Minocycline	$-N(CH_3)_2$	—H	—H

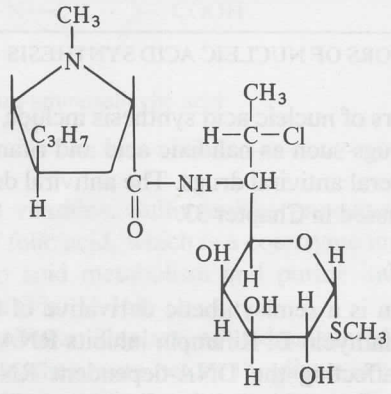
*There is no —OH at position 6 on methacycline and doxycycline.

Fig. 11-11. Structure of chloramphenicol.



Chloramphenicol

Fig. 11-12. Structure of clindamycin.



with inhibition of protein synthesis in bone marrow cells of the host. The drug gives rise to conditions such as anemia and leukopenia, and repeated administration may result in fatal aplastic anemia. It is still a drug of choice for typhoid fever, and for cases of meningitis in which the patient is allergic to penicillin.

Erythromycin

Erythromycin is most active against gram-positive species and is one of the least toxic of all antibiotics. It is a frequent alternative drug when other drugs are too toxic or when an individual is allergic to drugs such as the penicillins. It is the drug of choice in the treatment of infections caused by *Legionella pneumophila* and *Mycoplasma pneumoniae*.

Erythromycin affects protein synthesis by binding to free ribosomes but not polysomes. The ribosome-bound drug allows the formation of a small peptide but then prevents further elongation of the peptide.

Lincomycin and Clindamycin

Lincomycin and clindamycin resemble erythromycin in spectrum of activity and pharmacologic characteristics. Clindamycin (Fig. 11-12) is a derivative of lincomycin. Clindamycin was used routinely for the treatment of infections caused by the anaerobe *Bacteroides fragilis*; however, resistant isolates are now becoming more common. Clindamycin is still the drug of choice in the treatment of serious anaerobic pulmonary infections.

Clindamycin has a greater spectrum of activity than lincomycin, with activity against many gram-positive and gram-negative anaerobic bacteria as well as protozoal organisms such as *Toxoplasma* and *Plasmodium*. Diarrhea and pseudomembranous colitis are toxic side effects during clindamy-

cin therapy. These side effects are, however, controllable (see Chapter 21).

Lincomycin and clindamycin are similar to chloramphenicol in inhibiting protein synthesis, that is, they inhibit peptidyl transfer.

Spectinomycin

Spectinomycin is not a true aminoglycoside but is very similar structurally. It inhibits protein synthesis much as chloramphenicol does. Its principal use is in the treatment of gonorrhea when the agent of disease is resistant to penicillin.

INHIBITORS OF NUCLEIC ACID SYNTHESIS

Inhibitors of nucleic acid synthesis include antibacterial drugs such as nalidixic acid and rifampin but also several antiviral drugs. The antiviral drugs will be discussed in Chapter 33.

Rifampin

Rifampin is a semisynthetic derivative of the antibiotic rifamycin B. Rifampin inhibits RNA synthesis by affecting the DNA-dependent RNA polymerase of the bacterial cell. Mammalian RNA polymerases are not affected by the drug. Rifampin has activity against mycobacteria and gram-positive species but is less active against gram-negative species. It is used primarily in the treatment of tuberculosis, being combined with other drugs such as streptomycin and ethambutol. Most of the undesirable side effects of rifampin therapy are minor gastrointestinal symptoms.

Nalidixic Acid

Nalidixic acid is not related chemically to other antimicrobial agents (Fig. 11-13). It is most effective against gram-negative organisms but not anaerobes. Its use is restricted to very specific situations. It has been used in combination with other drugs to prevent infections in cancer patients undergoing therapy. The drug selectively suppresses those bacteria in the intestinal tract that are major causes of infection in the compromised cancer patient but does not affect the anaerobic population. Nalidixic acid is also a secondary drug in the treatment of shigellosis, a diarrheal disease.

Fig. 11-13. Structure of nalidixic acid.

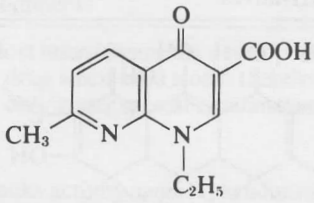
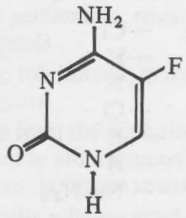


Fig. 11-14. Structure of flucytosine (5-fluorocytosine).



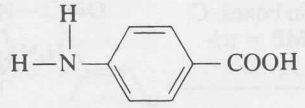
The bacteriostatic activity of the drug is associated with its ability to hinder DNA replication, presumably because of its effect on one of the DNA-binding proteins, DNA gyrase.

Flucytosine

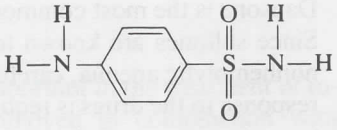
Flucytosine is a fluorinated pyrimidine (Fig. 11-14) originally developed as an antimetabolite for use in cancer chemotherapy. It has a narrow spectrum of activity that is limited to yeast infections such as candidiasis. When used alone it induces the development of resistant microbial species. It is frequently combined with amphotericin B for the treatment of systemic mycoses. It is rapidly absorbed from the intestinal tract and can be administered orally.

Flucytosine is deaminated in the cytoplasm of the fungal cell to 5-fluorouracil, which is subsequently phosphorylated and incorporated into RNA. This fluorinated derivative interferes with normal protein synthesis.

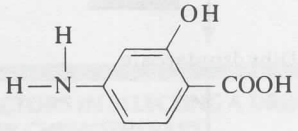
Fig. 11-15. Structural relationship between the antimetabolites sulfanilamide and para-aminosalicylic acid and the metabolite para-aminobenzoic acid.



Para-aminobenzoic acid
(PABA)



Sulfanilamide



Para-aminosalicylic acid
(PAS)

Toxic effects during therapy are minimal and include mild nausea, vomiting, and diarrhea.

ANTIMETABOLITES

Compounds that are structurally similar to normal cellular metabolites and may compete with them for attachment to an enzyme surface are called *antimetabolites*.

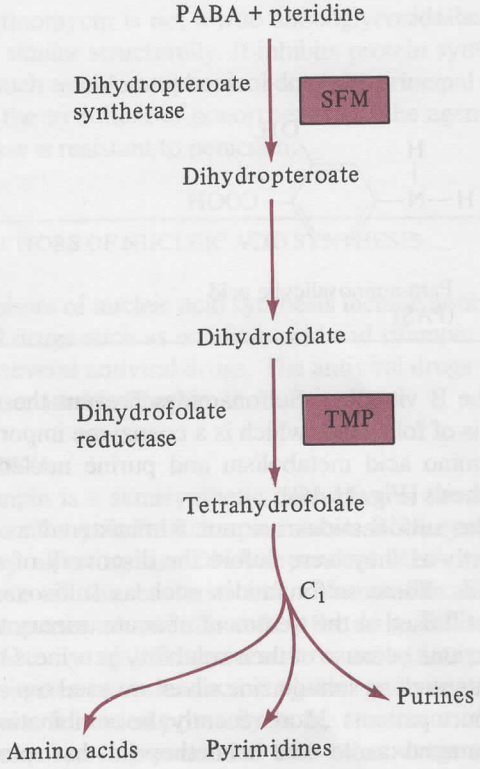
Sulfonamides

The sulfonamides were among the first synthetic agents used successfully in the treatment of disease. Important derivatives of the sulfonamides are sulfanilamide, sulfadiazine silver, sulfamethoxazole, and sulfamerazine. On account of their broad antibacterial activity these drugs were in earlier times used almost exclusively in the treatment of a wide assortment of diseases. It is most fortunate that other drugs have supplanted the sulfonamides as antimicrobial agents because all pathogenic bacteria are capable of developing resistance to the sulfonamides. The bacteriostatic action of the sulfonamides is related to their similarity in structure to para-aminobenzoic acid (PABA) (Fig. 11-15). PABA is a component of the vitamin folic acid, one of the B vitamins. Sulfonamides prevent the synthesis of folic acid, which is a coenzyme important in amino acid metabolism and purine nucleotide synthesis (Fig. 11-16).

The sulfonamides are not administered as frequently as they were before the discovery of antibiotics. Some sulfonamides such as sulfisoxazole are still used in the treatment of acute urinary tract infections because of their solubility in urine. Other agents such as sulfadiazine silver are used topically for burn patients. More recently the combination of sulfamethoxazole and trimethoprim has proved particularly effective against many intestinal and urinary tract pathogens. The reason for this effectiveness is the apparent inhibition of two reactions in the synthesis of folic acid (Fig. 11-16).

The sulfonamides are for the most part readily tolerated; however, they do have some undesirable side effects. The drugs can bind to plasma proteins that are normally occupied by bilirubin. In the newborn, bilirubin can penetrate the blood-brain barrier, causing encephalopathy (kernicterus). Sulfonamides should therefore not be administered to pregnant women. Approximately 1 to 3 percent of patients receiving sulfonamide therapy exhibit hypersensitivity to the drug. Persons with glucose 6-phosphate dehydrogenase deficiency experience a

Fig. 11-16. Reaction pathway in which para-aminobenzoic acid (*PABA*) is involved in the synthesis of amino acids, pyrimidines, and purines. The drugs that act as antimetabolites in the pathway are enclosed in boxes. C_1 = one carbon unit. (SFM = sulfonamide; TMP = trimethoprim; C_1 = one carbon unit.)

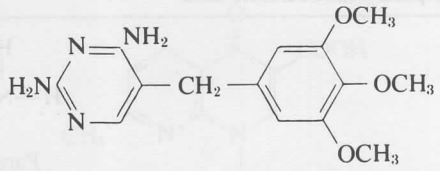


minor anemia caused by the instability of red blood cells. The anemia is severely exaggerated if they are treated with sulfonamides or nitrofurans.

Para-aminosalicylic Acid and the Sulfones

Both para-aminosalicylic acid (PAS) and the sulfones are analogues of PABA. PAS shows little activity against most organisms except *Mycobacterium tuberculosis*. It was once used in combination with other drugs for the treatment of tuberculosis, but today drugs such as ethambutol and rifampin have supplanted it in treatment. The sulfones are used almost exclusively in the treatment of leprosy.

Fig. 11-17. Structure of trimethoprim.



Dapsone is the most commonly prescribed sulfone. Since sulfones are known to cause hemolytic and nonhemolytic anemia, careful monitoring of patient response to the drugs is required during treatment.

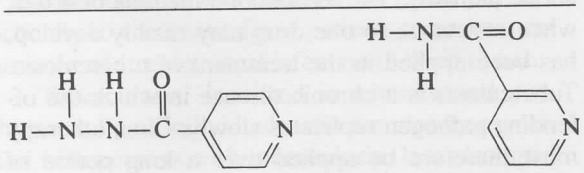
Trimethoprim

Trimethoprim is a structural analogue of pteridine (Fig. 11-17), a component of the folic acid molecule. It is a potent inhibitor of the enzyme dihydrofolate reductase in bacteria and interferes competitively with the conversion of dihydrofolic acid to tetrahydrofolic acid (see Fig. 11-16). The drug is not used alone but in combination with sulfamethoxazole, the combination called *co-trimoxazole*. This combination is bactericidal and has been used in the treatment of many types of diseases including urinary tract infections and respiratory and gastrointestinal diseases. The drug combination has proved very useful in the treatment of diseases refractory to the usual antibiotics. Serious toxicity is a rare event, but when it occurs the most likely complications are severe skin lesions and the combination of thrombocytopenia and leukopenia. Cotrimoxazole should not be administered to pregnant women or those known to be sensitive to sulfonamides.

Ethambutol

Ethambutol is used exclusively in the treatment of tuberculosis. It affects only growing microorganisms and is believed to interfere with RNA synthesis. It is beneficial in combination with other drugs for the treatment of tuberculosis because it helps delay the emergence of drug-resistant strains. At high dosage it can affect visual acuity.

Fig. 11-18. Structural relationship between isoniazid and nicotinamide.



Isoniazid

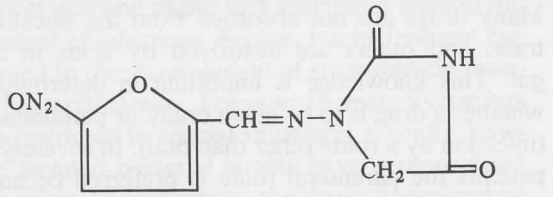
Isoniazid (INH) is important in the treatment of tuberculosis. It is employed in combination with other drugs such as streptomycin or rifampin. Isoniazid is particularly effective in treatment of tuberculosis because it is readily absorbed from the intestinal tract and is only mildly toxic to the host in low dosages. It resembles nicotinamide (Fig. 11-18) in structure and competes with it for incorporation into nicotinamide-adenine dinucleotide (NAD), an important coenzyme in metabolism.

Isoniazid is relatively nontoxic when administered in small doses. In 50 percent of the population (only 15 percent in Orientals) it is inactivated very slowly in the body and can result in a cumulative toxicity causing neuritis. The latter can be prevented by simultaneous administration of pyridoxine.

Nitrofurans

Nitrofurans have various antibacterial, antiprotozoal, and antifungal activities. They are bactericidal and bacteriostatic and are used primarily in the treatment of urinary tract infections. Nitrofurantoin (Fig. 11-19) is the most widely prescribed of the nitrofurans. Although its exact mechanism of action is not known it is believed to affect carbohydrate metabolism in the bacterial cell by interfering with acetyl coenzyme A activity.

Fig. 11-19. Structure of nitrofurantoin.



FACTORS IN SELECTING A DRUG FOR CHEMOTHERAPY

The choice of a drug is dictated primarily by the kind of microorganism (or microorganisms) involved in the infection. In some instances a direct relationship exists between the disease and the microorganism involved. Diphtheria, for example, is always caused by *Corynebacterium diphtheriae*. In other diseases, such as pneumonia, there may be more than one causal agent. The following factors must be considered before a drug is administered.

DOSAGE

The dosage of drug to be given to the patient may be affected by many factors, including the rate of excretion or inactivation of the drug, its toxic potential, the age of the patient, and the presence of underlying organic illness. Selecting the correct dosage depends on a knowledge of the minimal inhibitory concentration (MIC) of the drug (the smallest amount of antibacterial agent that still inhibits growth; see under Broth Dilution Test, later in this chapter) and the amount that will produce the MIC in the serum of the patient. The general recommendation is that the concentration of drug in the serum be three to five times the MIC to ensure effective therapy. In very serious infections the ratio of serum level to MIC may be as much as 100:1—for example, in penicillin therapy in meningitis infections caused by species of *Neisseria*.

ROUTE OF ADMINISTRATION

Many drugs are not absorbed from the intestinal tract, and others are destroyed by acids in the gut. This knowledge is important in determining whether a drug is to be given orally or parenterally (injection by a route other than oral). In severely ill patients the parenteral route is preferred because the drug will appear more rapidly at the site of infection and at higher concentrations than with the oral route. A great percentage of drug is excreted in the feces if it is given orally. Drugs not absorbed from the intestinal tract are effective in the treatment of gastrointestinal disorders when high concentrations of the drug are required.

DRUG COMBINATIONS

Drugs used in combination can produce a number of different results. (1) *Indifference* is a situation in which the combined effect of the two drugs is no greater than the effect of either one administered separately. (2) *Synergism* is a condition in which the activity of the two drugs together exceeds the combined activity of each drug administered separately. Synergism occurs frequently when two bactericidal agents are administered. In the treatment of bacterial endocarditis, for example, penicillin and streptomycin have an effect that neither drug alone can accomplish. (3) In *addition* the effect of the combined drugs is equal to the sum of the effects of each drug administered separately, and (4) in *antagonism* the combined effect is less than that of either drug administered separately. Antagonism is often incurred when a bactericidal and a bacteriostatic agent are given together; the bacteriostatic agent inhibits growth, and microbial growth must be present before some bactericidal agents (such as penicillin) are effective.

Combination drug therapy can be expensive and increase the risk of toxicity and superinfection. Still, combinations of drugs are of important clinical value. Enhanced bactericidal effects may result when (1) a cell wall–active agent is combined with an aminoglycoside, (2) a beta-lactam is combined with inhibitors of beta-lactamase, and (3) the combined drugs inhibit separate steps in a critical metabolic pathway. The use of combinations of drugs, when resistance to one drug may readily develop, has been applied in the treatment of tuberculosis. Tuberculosis is a chronic disease in which the offending pathogen replicates slowly. Chemotherapy must therefore be applied over a long period of time. The use of one drug for treatment invariably selects for resistant microbial strains.

Toxicity from a single drug can sometimes be reduced by using a smaller dose in combination with another chemotherapeutic agent. For example, amphotericin B dosage can be reduced by using it in combination with flucytosine in the treatment of fungal disease. Drug combinations are used primarily (1) in mixed infections, (2) to prevent emergence of drug-resistant strains, and (3) as a stopgap measure in bacteriologically obscure illness.

The inappropriate use of drug combinations can lead to serious consequences. One of the drugs may select for an organism carrying the drug resistance factor on a plasmid. The plasmid may also carry other antibiotic resistance genes, thus complicating chemotherapy.

PROPHYLAXIS

The prophylactic administration of drugs can sometimes be detrimental to patients, especially if prophylaxis is continued for several days. Continued exposure of the host's microbial flora to antimicrobials often selects for resistant strains, and this can lead to superinfection. Prophylaxis is recommended when the host is subjected to a treatment or therapy, not involving antimicrobials, that can lead to serious disease, as in the following circumstances:

1. Dental extraction. Chemotherapy is considered before dental extractions only if the patient has a history of rheumatic fever, subacute bacterial endocarditis, or congenital heart defect. Extraction leads to displacement of oral microorganisms, particularly species of streptococci, into the circula-

tion. These microorganisms can be implanted on heart tissue and can lead to serious disease.

2. Rheumatic fever. Penicillin is given prophylactically to rheumatic fever patients to prevent recurrence of streptococcal infection in heart tissue.

3. Tuberculosis. Isoniazid is administered prophylactically to individuals who are closely associated with persons with tuberculosis. It is also used to prevent the development of progressive tuberculosis.

4. Tonsillectomy. Prophylaxis is indicated for patients with a history of rheumatic fever, bacterial endocarditis, or congenital heart disease.

5. Surgical procedures. Antibiotic prophylaxis appears to be necessary only when there is a penetrating wound of the abdomen (for example, in bowel surgery, which presents a high probability of contamination) and in vaginal hysterectomy. Prophylaxis is usually directed at preventing staphylococcal infection. In surgical techniques that are considered "clean" (not likely to be contaminated), antibiotics are not recommended. Antibiotics should never be considered a substitute for careful surgical procedures.

TOXICITY

The toxic potential of a drug is a major factor in the selection of a chemotherapeutic agent, especially if the drug is to be administered to children, pregnant women, the elderly, and persons suffering from organic diseases. In patients with underlying renal disease antibiotics may persist in the body for extensive lengths of time because of the inability of the kidneys to excrete them. Polymyxin, amphotericin B, and bacitracin should be used with extreme caution since they are directly toxic to kidney tissue.

DRUG RESISTANCE

Since the first antibiotics were discovered over 50 years ago, there has been a continual race to find new antibiotics or derivatives before microorganisms develop resistance to the older drugs. Streptomycin was one of the first antibiotics used in the treatment of infectious disease, but its frequent use soon led to the development of streptomycin-resistant bacterial strains. For over 15 years its use has been restricted to special situations; recently, however, isolated bacterial strains have demonstrated their original sensitivity to the drug. Antimicrobial resistance is due to genetic changes in microorganisms. The single most important feature of genetic resistance to antimicrobials is the presence of plasmids and transposons in the bacterial cell. Mutation alone is usually a very ineffective way to bring about resistance because only a single trait is involved. The plasmid, however, can carry determinants making the organism resistant to multiple agents. The ability of plasmids to be transported from one species to another and to be transported by virus following integration into the viral genome makes them important mediators of resistance. Transposons also carry antibiotic resistance determinants and facilitate transfer between plasmids, between plasmids and chromosomes, and between plasmids and bacteriophages. What makes transposons so important is their ability to move from one DNA molecule to another without the usual strict recombination requirements that are associated with chromosomal DNA-DNA interactions.

Four major mechanisms enable microorganisms to resist the action of antimicrobials: interference with transport across the cell wall or cytoplasmic membrane, enzymatic modification of the drug, alteration of the antimicrobial target, and synthesis of resistant metabolic pathways.

INTERFERENCE WITH TRANSPORT

Interference with antimicrobial transport is associated with chromosomal mutations. Such mutations result in alterations of the structure of cell envelope components or changes in certain sugar transport systems. Alterations in the lipid content of the cell wall of species of *Pseudomonas* have been demonstrated to prevent the entry of certain antimicrobials.

Located in the periplasmic space of some microorganisms are enzymes that modify and inactivate an antibiotic and prevent its transport across the cytoplasmic membrane into the cytoplasm. Chloramphenicol, for example, is modified and inactivated by chloramphenicol acetyltransferase and is not transported into the cytoplasm of species of *Pseudomonas*.

Aminoglycosides have no effect on obligate anaerobes or facultative anaerobes that are grown anaerobically. The basis for this resistance is that antibiotic transport requires an effective electrical potential across the membrane. Without this membrane potential an effective proton motive force is not generated and the antibiotic is not transported. Thus all anaerobes are resistant to aminoglycosides even though their ribosomes are sensitive to these antibiotics.

ENZYMATIC INACTIVATION

The most important antimicrobial resistance mechanism in bacteria is plasmid-mediated enzymatic inactivation (Table 11-3). Most of the enzyme inactivators are located in the periplasmic space. They include the beta-lactamases (beta-lactamases of *S. aureus* are excreted extracellularly; the same enzymes of gram-negative organisms are found in the periplasmic space) and enzymes that acetylate, adenylate, or phosphorylate various aminoglycosides. The only drug-inactivating enzyme located in the cytoplasm is chloramphenicol transacetylase. Modification occurs at a specific amino or hydroxyl group on the drug. Some drug-modification sites are common to many of the aminoglycosides, and thus cross resistance is common. The manner in which modification of aminoglycosides renders them inactive is not known but may be the result of (1) competition with unmodified aminoglycosides, (2) ineffective binding to ribosomes, or (3) inability to interfere with ribosome function. A number of aminoglycoside resistance determinants are found on transposable elements. In some instances the transposon resistance factor is associated with as many as four or five resistance determinants. Thus a wide spectrum of cross resistance can be found in microorganisms when these transposons have been disseminated. It is the promiscuity of the transposon that makes antimicrobial therapy so difficult.

Table 11-3. Modifying Enzymes of the Aminoglycosides

Enzyme	Antibiotic substrate
Acetyltransferase	Kanamycin, tobramycin, amikacin, sisomicin, neomycin, gentamicin
Adenyltransferase	Tobramycin, amikacin, kanamycin, neomycin, gentamicin, streptomycin, spectinomycin
Phosphotransferase	Neomycin, kanamycin, streptomycin, gentamicin

ALTERATION OF THE MICROBIAL TARGET

Alteration of the microbial target is usually associated with a chromosomal mutation, but plasmids may also be involved. Two of the most widely examined instances involving alteration of a microbial target are penicillin-binding proteins and the 30S ribosome.

The catalytic actions associated with the various PBP's have already been discussed. If PBP's are absent or are altered in such a way that their affinity for beta-lactams is decreased then penicillin resistance may ensue.

Streptomycin resistance is associated with the loss or alteration of a single protein in the 30S ribosomal subunit. These mutations result in decreased ribosomal binding of streptomycin.

Other targets whose alteration results in resistance include DNA gyrase, resistance to nalidixic acid; RNA polymerase, resistance to rifampin; and methylated 23S RNA, resistance to erythromycin and lincomycin.

SYNTHESIS OF RESISTANT METABOLIC PATHWAYS

Synthesis of resistant metabolic pathways is associated with resistance to sulfonamides and related

antimetabolites. An altered enzyme allows the organism to bypass the competitive inhibition of these drugs (see Fig. 11-16). This type of resistance has been observed in the Enterobacteriaceae during treatment with trimethoprim. These organisms are known to contain plasmid-coded dihydrofolate reductases or dihydropteroate synthetases that are resistant to the drug.

QUANTITATIVE DETERMINATION OF ANTIBIOTIC ACTIVITY

Both qualitative and quantitative measurements of potential antibiotics must be made to determine their usefulness as chemotherapeutic agents in the treatment of human infections. In these procedures the unknown antibiotic is tested against microbial species such as *Escherichia coli, S. aureus*, and *Pseudomonas aeruginosa* that are fast growers and are known to be sensitive to antibiotics. The same tests are applied in the clinical setting to determine the susceptibility of an unknown pathogen, recently isolated from an infected patient, to known antibiotics. Susceptibility tests are important for evaluating which antibiotics can be used in the treatment of the disease.

BROTH DILUTION TEST

In the broth dilution test the minimal inhibitory concentration (*MIC*) and minimal bactericidal concentration (*MBC*) of the drug can be determined. Although an unknown drug can be tested against a number of microbial species, *E. coli* and *S. aureus* are generally employed as the standard test organisms. If one were measuring the effect of a known antibiotic on a pathogen isolated from a patient, *E. coli* and *S. aureus* would instead be used as controls because of their known susceptibility to the antibiotic and rapid rate of growth.

In the broth dilution test a standard inoculum of the test organism is seeded into tubes of sterile broth containing decreasing concentrations of the antibiotic (Fig. 11-20). After 24 hours of incubation at 35°C the MIC is determined by finding the lowest concentration of drug inhibiting bacterial growth. The MBC can be found by subculturing those tubes showing no visible growth into broth tubes that contain no antibiotic. Subculturing dilutes out the antibiotic. The lowest concentration of drug in the original culture that leads to no growth is the MBC. Major disadvantages of the broth dilution technique are that (1) only one organism can be run in a single series of tubes, (2) contamination is difficult to detect in broth, and (3) the test is expensive if large numbers of bacterial strains are being surveyed, although a microtiter assay has reduced costs considerably. Many laboratories prefer the agar dilution method discussed below.

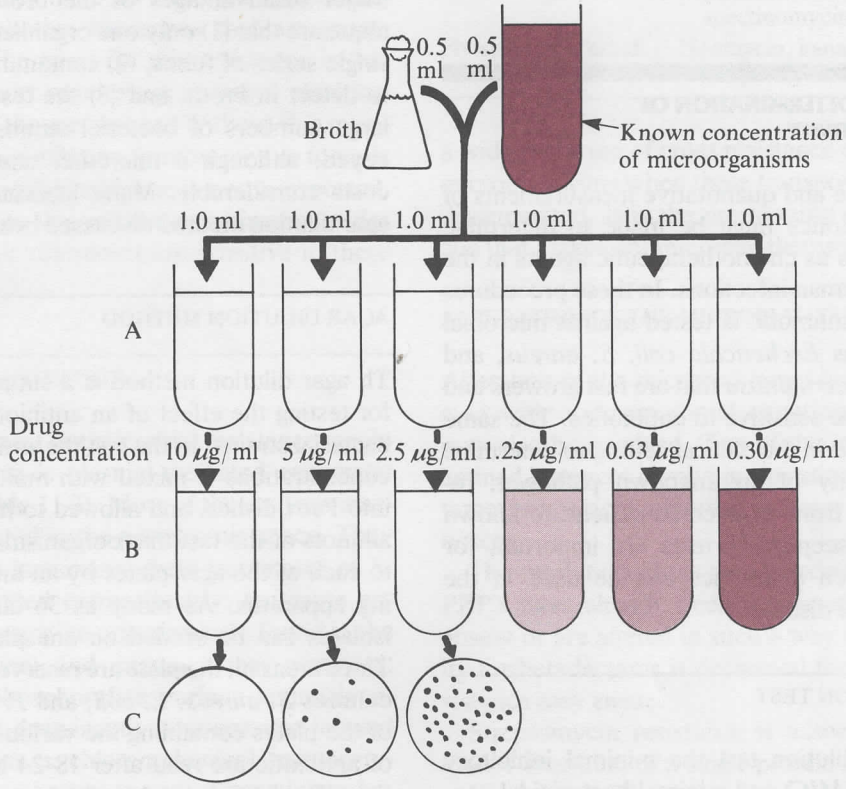
AGAR DILUTION METHOD

Th agar dilution method is a simplified procedure for testing the effect of an antibiotic against many bacterial strains. In the test the antibiotic at various concentrations is mixed with molten agar, poured into Petri dishes, and allowed to harden. Standard aliquots of the test microorganisms are transferred to each of the agar plates by an inoculum-replicating apparatus. As many as 36 different bacterial isolates can be seeded on one plate (Fig. 11-21). Three areas on the plate are reserved for the control cultures *S. aureus, E. coli*, and *P. aeruginosa*. All of the plates containing the various concentrations of antibiotic are read after 18–24 hours of incubation. The MIC is read as the lowest concentration of antibiotic that inhibits growth. Both the broth and agar dilution techniques yield more precise results, but because of heavy work loads most laboratories use the disk diffusion test.

DISK DIFFUSION TEST

In the disk diffusion test, paper disks impregnated with known amounts of antibiotics are placed on an agar medium seeded with a known species of bacteria. As the antibiotic diffuses into the medium, it

Fig. 11-20. Broth dilution test used to determine minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) of antimicrobial compounds. A. Each broth tube contains 9.0 ml of sterile broth with a different concentration of antimicrobial. To each tube is added 0.5 ml of broth and 0.5 ml of a known concentration of microorganisms. The concentration of microorganisms in each broth tube is so small that there is no visible turbidity. Tubes are incubated at 35°C for 12–18 hours. B. Tubes are examined for macroscopic growth. Lowest concentration of drug showing no growth is the MIC. In this case it is 2.5 μg/ml. C. To determine MBC, loopfuls are removed from tubes showing no visible growth and are streaked on agar plates. Those plates showing no growth indicate drug concentrations that destroy the microorganisms (10 μg/ml).



produces a gradient of antibiotic concentration. After suitable incubation antibiotic activity is determined by the width of the zone of no growth around the antibiotic disk (Fig. 11-22).

Although basically a qualitative test, the disk diffusion test can be made quantitative in the following way. The size of the zone of inhibition can be correlated with the clinical susceptibility of the microorganism to the antibiotic, provided standardized conditions are maintained. One standardized test is called the *Kirby-Bauer* method. In this technique, Mueller-Hinton agar, a standard inoculum, and a single antibiotic disk of standardized potency (usually 30 μg for many antibiotics) are used. Zones of inhibition are described as susceptible, intermediate, and resistant. The breakpoint between the zones for each antibiotic is obtained from published tables. The Kirby-Bauer tables have taken into account the MIC of the organism, the blood levels obtained with normal dosage of the anti-

Fig. 11-21. Steers inoculum-replicating device. Plate on left contains 36 reservoirs for bacterial inocula. Bottom plate on right contains antibiotic-containing medium on which bacteria will be seeded. (From J. A. Washington, II, *Mayo Clin. Proc.* 44:811, 1969.)

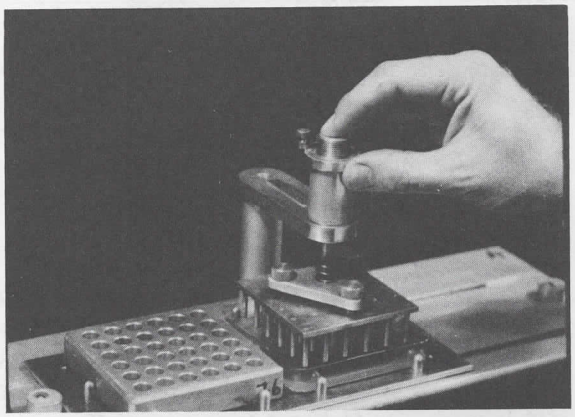
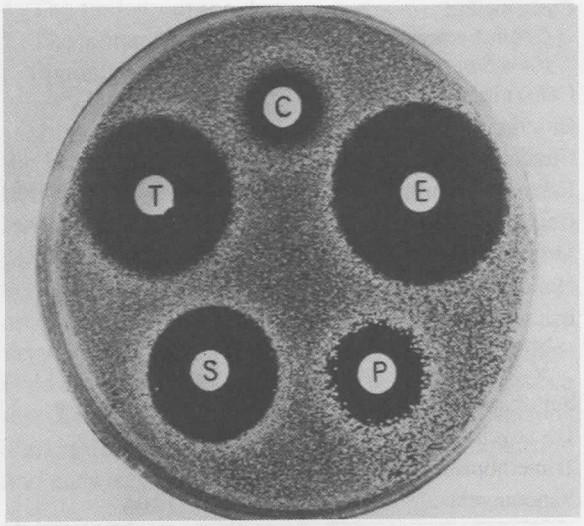


Fig. 11-22. Disk diffusion test. An agar medium is inoculated with a strain of *Staphylococcus aureus*. Disks containing specified amounts of antibiotic are placed on the agar. The type of antibiotic in each disk is indicated by the letter: *T*, tetracycline; *C*, chloramphenicol; *E*, erythromycin; *P*, penicillin; *S*, streptomycin. Inhibition of growth of *S. aureus* is evidenced by a clear zone around the antibiotic disk. (From L. P. Garrod and F. O'Grady, *Antibiotic and Chemotherapy* [5th ed.]. London: Churchill Livingstone, 1973.)



biotic, and the distribution of zone sizes among species with known responsiveness to the antibiotic (Table 11-4).

ANTIBIOTIC CONCENTRATIONS IN THE PATIENT'S SERUM

An estimate of the concentration of an antibiotic in the patient's serum or other body fluids is necessary when very toxic antimicrobials are being administered. Depending on the health of the patient and the condition of various organs such as the kidneys, the concentration of the drug in the serum may be at or above toxic levels during the course of therapy. Under these circumstances the serum should be assayed periodically. One commonly employed technique for serum assay is the agar diffusion technique, used often for gentamicin assay. In this procedure a patient's serum is removed at times when the antimicrobial will be at maximum and zero levels. An agar plate seeded with *B. subtilis* spores is prepared. Normal serum (not from the patient) is placed on disks containing standard amounts of the antimicrobial. The test serum from the patient is then placed on disks. The standard and the test disks are placed on the agar plate, which is incubated. After 2–3 hours zones of inhibition around the disks containing the antimicrobial are observed. A standard curve is produced relating concentrations of antibiotic to zones of inhibition. The zones of the unknown samples are also determined, and by extrapolation from the standard curve the concentration of the antimicrobial in the patient's serum is found. Other methods available for assay of antimicrobials include immunological assays, enzymatic assays, chromatographic assays, and chemical assays.

Table 11-4. Zone Size Interpretative Standards for Some Antimicrobial Agents Used in the Treatment of Infectious Disease

Antimicrobial agent	Disk content	Inhibition zone diameter (to nearest mm)		
		Resistant	Intermediate	Sensitive
Amikacin	30 μg	≤14	15–16	≥17
Ampicillin				
Gram-negative enterics	10 μg	≤11	12–13	≥14
Staphylococci	10 μg	≤28	—	≥29
Carbenicillin				
Enterobacteriaceae	100 μg	≤17	18–22	≥23
Pseudomonas	100 μg	≤13	14–16	≥17
Cefoxitin	30 μg	≤14	15–17	≥18
Chloramphenicol	30 μg	≤12	13–17	≥18
Clindamycin	2 μg	≤14	15–16	≥17
Erythromycin	15 μg	≤13	14–17	≥18
Gentamicin	10 μg	≤12	13–14	≥15
Moxalactam	30 μg	≤14	15–22	≥23
Nalidixic acid	30 μg	≤13	14–18	≥19
Penicillin G				
Staphylococci	10 units	≤28	—	≥29
N. gonorrhoeae	10 units	≤19	—	≥20
Sulfonamides	250 or 300 μg	≤12	13–16	≥17
Tetracycline	30 μg	≤14	15–18	≥19
Trimethoprim	5 μg	≤10	11–15	≥16
Vancomycin	30 μg	≤ 9	10–11	≥12

Source: From M2-T3, *Performance Standards for Antimicrobial Disk Susceptibility Tests* (3rd ed.). Villanova, PA: National Committee for Clinical Laboratory Standards, 1983.

SUMMARY

Chemotherapeutic agents are used in the treatment of disease and are basically of two types: antibiotics produced by microorganisms and synthetic agents produced in the laboratory. Once an antibiotic has been isolated from the soil or synthesized in the laboratory, several characteristics must be evaluated before the drug can be considered for human use. The most important consideration is that the drug should exert antimicrobial activity at high dilutions and be relatively nontoxic to host tissue. Other factors to consider are

1. Solubility of the drug in water
2. Stability of the drug at different temperatures
3. Excretion rate of the drug from the kidneys
4. Permeability of the drug into various tissues
5. Hypersensitivity of the host to the drug
6. How many species or groups of microorganisms the drug is active against
7. How rapidly drug-resistant forms emerge once the microorganism has been exposed to the drug
8. Degree of toxicity to host tissue

Chemotherapeutic agents either kill the organism or inhibit its growth. They affect the microbial cell in different ways:

1. Inhibitors of cell wall synthesis, including peni-

cillins, cephalosporins, bacitracin, and vancomycin
2. Inhibitors of cytoplasmic membrane function, including polyenes, imidazoles, and polymyxins
3. Inhibitors of protein synthesis, including the aminoglycosides, tetracyclines, chloramphenicol, erythromycin, lincomycin, clindamycin, and spectinomycin
4. Inhibitors of nucleic acid synthesis, including nalidixic acid, rifampin, and flucytosine
5. Antimetabolites, including sulfonamides, para-aminosalicylic acid, trimethoprim, ethambutol, isoniazid, and nitrofurans

Microorganisms develop resistance to drugs because of genetic changes in the cell. Most antimicrobial resistance is due to the presence of plasmids and transposons that carry antibiotic resistance determinants readily transferred to other cells. Several mechanisms exist for antibiotic resistance in the cell:

1. Alteration of the target site
2. Drug modification resulting in inefficient transport
3. Synthesis of resistant metabolic pathways
4. Enzymatic modification and inactivation of drugs

Most resistance to antimicrobials is via drug-inactivating mechanisms.

Several factors must be taken into consideration before a drug is used for chemotherapy:

1. The dosage
2. The route of administration
3. Whether a combination of drugs should be used
4. The toxicity of the drug
5. The potential for the drug to induce the development of drug-resistant strains

Qualitative and quantitative tests are available in the laboratory to determine an organism's susceptibility to various antibiotics. It is important to determine the relative susceptibility of the pathogen to a drug before the latter is administered to prevent or cure disease. Three types of tests are available. The broth dilution and agar dilution tests are very accurate but also time-consuming. In the clinical laboratory the disk diffusion test is a qualitative test that has been made quantitative by various standardizations. This test is relatively inexpensive and easy to carry out.

QUESTIONS FOR STUDY

1. Once an antibiotic has been isolated from the soil-inhabiting microorganism, what two factors are most important in deciding whether to continue further studies of the drug?
2. Where are the penicillin-binding proteins located in bacteria, and what are their functions, if any?
3. What is the clinical advantage of cephalosporins over penicillins?
4. Why are polyenes selectively toxic?
5. Make a list of the most toxic chemotherapeutic agents and define their modes of toxicity.
6. What are the two ways streptomycin affects protein synthesis?
7. What are the conditions under which tetracyclines should not be administered?
8. Under what conditions is it necessary to administer chemotherapeutic agents prophylactically?
9. In general, in what circumstances is it necessary to administer drugs in combinations?
10. What is the most common mechanism of drug resistance in bacteria?
11. What components in the bacterial cell are sub-

ject to microbial alteration that enables them to resist the action of antimicrobials?

12. What is the advantage (or advantages) of the broth and agar dilution tests for microbial drug susceptibility over the disk diffusion test? Disadvantages?
13. When is it necessary to determine the level of certain antibiotics in the patient's serum?
14. How does one determine the MIC of a drug? The MBC of the drug?

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12. INTERACTION BETWEEN HOST AND INFECTIOUS AGENT

OBJECTIVES

To differentiate between a commensal and a parasite; an intracellular and extracellular parasite; an infection and a disease; pathogenicity and virulence

To describe the various host factors present on skin, mucosal surfaces, respiratory tract, intestinal tract, genitourinary tract, and epithelial and subepithelial surfaces that resist invasion by microorganisms

To briefly explain how race, species, nutrition, occupation, and underlying conditions affect the host's resistance to disease

To list the major microbial toxins and their mechanism of action in the host

To briefly explain the distinctions between exotoxins and endotoxins

To describe the effects of endotoxin on the host

To explain the importance of bacterial adherence in the infectious disease process and the examples in which it is most evident

To define: indigenous, attenuation, invasive, systemic, lactoferrin, siderophore, transferrin, enterotoxin, toxoid, vaccine, adhesin

Microorganisms as Parasites
Extracellular Parasitism
Intracellular Parasitism
Terminology Associated with the Infectious Disease Process
Host Factors and the Disease Process
Portal of Entry
Skin
Mucosal Surfaces
Respiratory Tract
Oropharynx
Intestinal Tract
Urogenital Tract
Epithelial and Subepithelial Factors
Resistance
Species
Race
Nutrition
Occupation
Underlying Conditions
Microbial Factors Contributing to Infectious Disease
Invasiveness of Bacteria
Toxins
Exotoxins
Endotoxins
Antiphagocytic Factors
Bacterial Adherence

One of the most important areas of microbiological research involves the molecular biology of the host-parasite interaction. The initial step in the host-parasite interaction often is the binding of microbe to host tissue. A knowledge of the chemistry and molecular biology of the components involved in this process will eventually lead to the development of vaccines to prevent disease. The purpose of this chapter is to describe those factors, both microbial and host-related, that are associated with the disease process.

MICROORGANISMS AS PARASITES

A parasite is an organism that lives on or within a host, deriving nutrients from it. The human body is a breeding ground for a wide variety of parasites, particularly the bacteria. In certain areas of the body they are found in appreciable numbers, as in the upper respiratory tract, lower intestinal tract, and skin. In other areas, such as the esophagus, urinary tract, and stomach, microorganisms are few or nonexistent. The normally sterile areas of the body include the blood, spinal fluid, urine, and endothelial tissues. The factors that determine the kind and number of microorganisms at any one site in or on the host are (1) the availability or unavailability of oxygen, (2) the pH of the host site, (3) the influence exerted by other microorganisms, (4) the availability of nutrients, (5) the immunological response of the host to the presence of the microorganism, and (6) the availability of appropriate receptor sites. To be a successful parasite the microorganism must gain entrance into the host and must be able to establish a stable relationship with the host. The relationship should be such that neither host nor parasite is harmed. Such a relationship is referred to as *commensalism*. In addition the parasite must be able to survive the transit from one host to another if the host dies. The invading parasite may or may not harm the host, depending on a number of environmental and host factors that are discussed later in the chapter. Once microorganisms invade the host, they take up an extracellular or intracellular habitat.

EXTRACELLULAR PARASITISM

Most pathogenic species of bacteria exist as extracellular parasites in the infected host, that is, the invading microorganisms are not harbored nor do

they multiply within cells of the host. The damage inflicted on the host by extracellular parasites is usually caused by the production of bacterial enzymes, toxins, or low molecular weight molecules. These factors will be discussed in more detail later in the chapter.

INTRACELLULAR PARASITISM

Few microorganisms other than viruses can exist as intracellular parasites. Those that do must resist digestion by the host cell if this kind of parasitism is to continue. Some intracellular parasites are facultative and can replicate inside or outside the host cell. Others are obligate and can survive only within the confines of the host cell. Examples of facultative intracellular parasites are *Salmonella* species, *Mycobacterium tuberculosis*, species of *Brucella*, and certain fungi, including *Histoplasma capsulatum*. These parasites are not absolutely dependent on intracellular metabolites for their growth and development. Examples of obligate intracellular parasites are the rickettsiae, chlamydiae, *Mycobacterium leprae*, viruses, and certain protozoa such as the malaria parasite. The rickettsiae lack many of the glycolytic enzymes necessary for metabolism, while the viruses have no biosynthetic machinery and require those of the host to replicate. The protozoon *Plasmodium vivax*, which causes malaria, is unable to synthesize many coenzymes and thus requires the intracellular environment of the red blood cell to compensate for this deficiency. Inside the host cell, whether it be a lymphocyte, a red blood cell, or some other tissue cell, the infecting agent must reach an equilibrium with the host cell to survive. The infecting agent, therefore, must regulate its own growth so that it does not overcompete for cellular metabolites and thus starve and kill the host. Further, the infecting agent must resist digestion by the host cell. Many bacterial cells ingested by phagocytes, for example, are destroyed by lysosomal enzymes. Intracellular parasites resist digestion by mechanisms and thus thwart the natural defense measures of the host.

TERMINOLOGY ASSOCIATED WITH THE INFECTIOUS DISEASE PROCESS

The terms *infection* and *disease* are often used synonymously when discussing host-parasite relationships. To better comprehend the difference between these terms it is helpful to understand the development of the normal microflora, which represents a successful host-microorganism relationship.

Before birth the fetus is essentially a sterile environment, but at delivery microorganisms come into contact with the host. Some of these microorganisms are derived from the mother or others who come into contact with the infant. Some of these microorganisms are transient passersby that are destroyed by conditions in the host, but others establish themselves and produce microcolonies. This latter group has therefore colonized the host. As several types of microorganisms colonize a particular habitat the *microflora* is established (Fig. 12-1). This indigenous microflora will remain with the host for life, with only minor changes resulting from disease, dietary alterations, or hormonal changes. Many of the indigenous microorganisms appear to be important in maintaining the health of the host. For example, in some animals microorganisms of the intestinal tract are capable of synthesizing vitamins such as pantothenic acid, riboflavin, and vitamin B_{12}. It has been suggested that certain vitamin deficiencies in the human diet can be remedied by bacterial vitamin synthesis in the intestinal tract. Experiments on germ-free animals have also demonstrated that without an intestinal microflora there is a reduction in the size and plasticity of the digestive tract, compared with that in conventional animals. The indigenous microflora represents a commensalistic relationship with the host. Commensals live in a dynamic and harmonious balance with their host, but as we shall see sometimes this balance is upset and disease ensues.

A microorganism that makes contact with the host but does not cause the host to produce an immune response that would destroy it or cause an

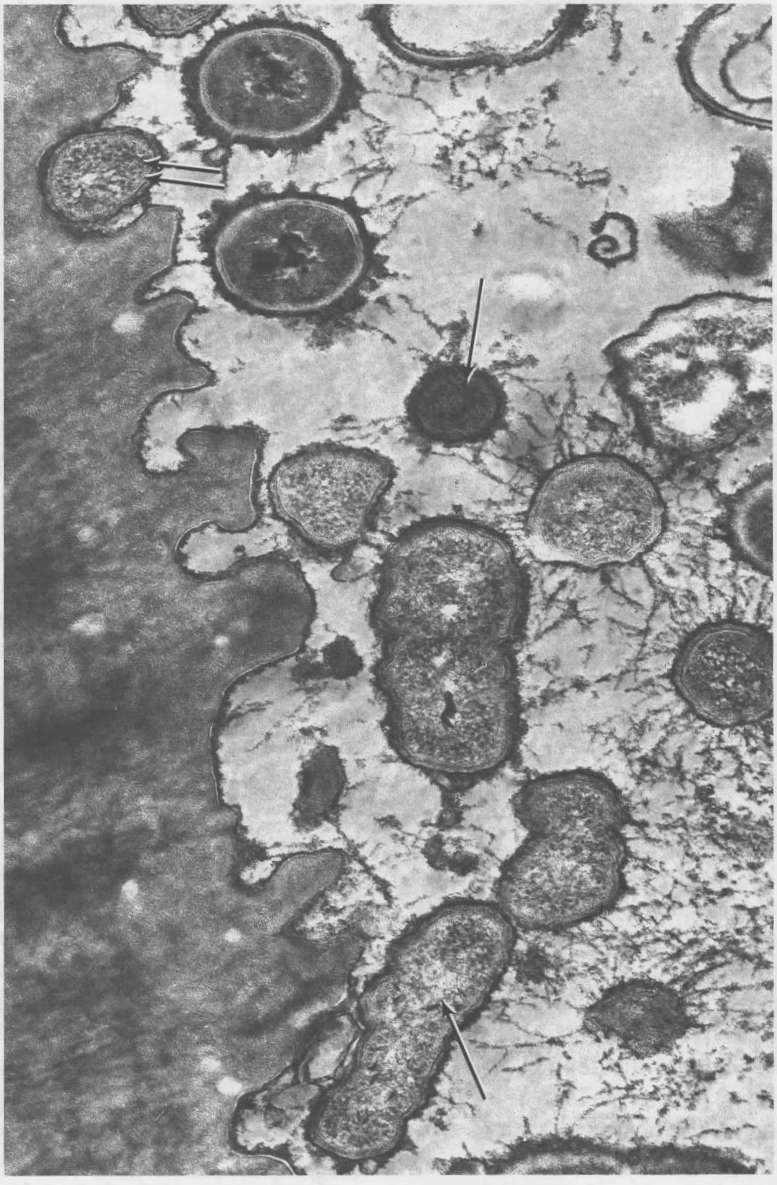


Fig. 12-1. Microflora of the bovine rumen (stomach). Microorganisms (*arrows*) are rod-shaped or coccal, and some are in process of cell division. One microorganism (*double arrow*) is firmly attached to the intestinal epithelium. The lacy material observed around some microorganisms is extracellular polymeric substances that take up the special stain (ruthenium red) used in the preparation. (From J. W. Costerton, et al., *Annu. Rev. Microbiol.* 35:299, 1981.)

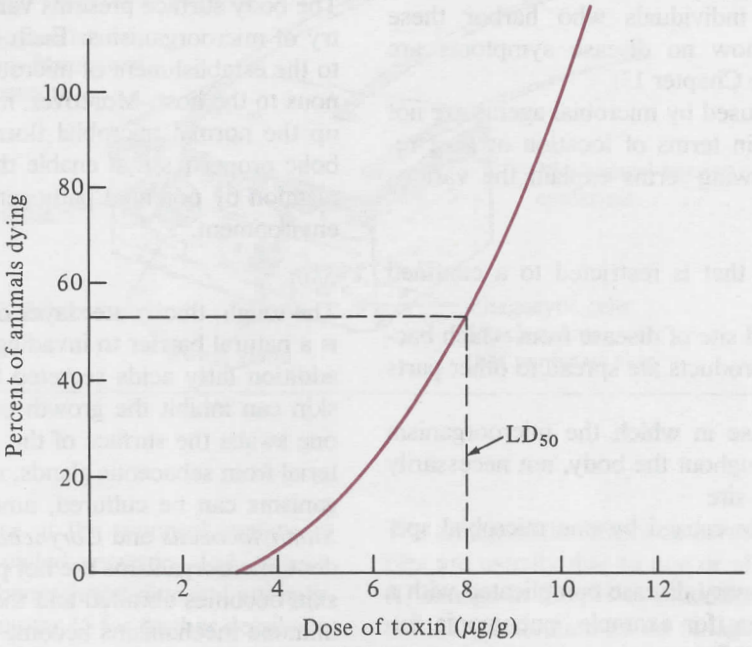


Fig. 12-2. Determination of the 50 percent lethal dose (LD_{50}) for a toxin. Various doses of toxin are injected into animals. The percentage of animals that die is plotted and the LD_{50} extrapolated. In this figure 8 μg of toxin per gram of animal is the LD_{50}.

allergic response is called a *colonizer*. Microorganisms that make contact with the host and elicit an immune response (antibody production) are said to cause an *infection*. If the microorganism brings about an abnormal condition in the host, in addition to eliciting antibodies, it is said to cause a *disease*. It is conceivable that a microorganism indigenous to the host could cause disease if the host were compromised by some underlying condition (e.g., diabetes) or treatment (antibiotic therapy) that caused the commensal to become a parasite.

Microorganisms that cause disease are said to be *pathogenic*, but some microorganisms are more likely to be pathogens than others. The term *virulence* refers to a quantitative measurement of a microorganism's pathogenicity. In other words one can measure the number of microorganism required to cause disease or the number of microorganisms that can cause death in 50 percent of the test animals: the infectious dose, ID_{50}, or lethal dose, LD_{50}, respectively (Fig. 12-2). Toxin produced by a microorganism can, in some instances, be used in these measurements. Virulence is not always a stable property; it can be modified in the laboratory. A highly virulent microorganism may lose its virulence on serial passage of the microbe in unnatural hosts. Growing the microorganism in laboratory media devoid of certain compounds may also cause it to lose its virulence. The loss or substantial reduction of virulence is referred to as *attenuation*. In addition some bacteria are pathogenic because they harbor certain bacterial viruses that carry information for toxin production. When the bacterial cell is cured of these viruses, the bacterium becomes avirulent. The virulence of bacteria, or their ability to cause disease, is related to their capacity either to invade tissue (*invasiveness*) or to produce toxins that affect host tissue. Among the literally thousands of microbial species, probably no more than 300 can cause disease in humans

uations in which a highly pathogenic microorganism is not a pathogen. For example, some individuals because of certain inherited genetic traits can be infected by a pathogen but show no symptoms of disease. The individuals who harbor these pathogens and show no disease symptoms are called *carriers* (see Chapter 13).

The diseases caused by microbial agents are not always the same in terms of location or host response. The following terms explain the various types of disease:

LOCAL a disease that is restricted to a confined area

FOCAL a localized site of disease from which bacteria and their products are spread to other parts of the body

SYSTEMIC a disease in which the microorganism can spread throughout the body, not necessarily from a localized site

PRIMARY a disease caused by one microbial species

SECONDARY a primary disease complicated with a second pathogen (for example, pneumonia following primary influenza)

MIXED a disease caused by two or more microorganisms

INAPPARENT OR SUBCLINICAL a disease that does not give rise to any detectable, overt manifestations

LATENT a disease that persists in the tissues in a dormant state and later becomes manifest, usually when there is lowered host resistance (also see Chapters 13 and 33)

BACTEREMIA a transitory disease in which bacteria present in the blood are usually cleared from the vascular system with no harmful effects

SEPTICEMIA a condition in which the blood serves as a site of bacterial multiplication as well as a means of transfer of the infectious agent from one site to another

PYEMIA the presence of pyogenic (pus-forming, such as staphylococci and streptococci) bacteria in the blood as they are being spread from one site to another in the body

TOXEMIA the presence of microbial toxins in the blood

HOST FACTORS AND THE DISEASE PROCESS

PORTAL OF ENTRY

The body surface presents various sites for the entry of microorganisms. Each site offers resistance to the establishment of microorganisms not indigenous to the host. Moreover, microorganisms make up the normal microbial flora and possess metabolic properties that enable them to prevent colonization by potential pathogens acquired from the environment.

Skin

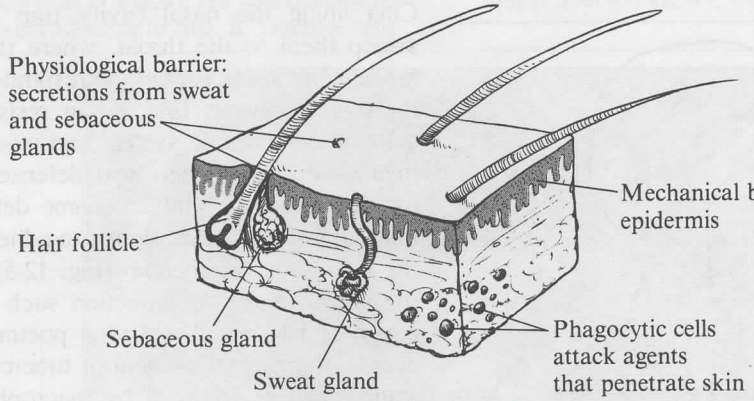
The tough, thick outer layer of the skin (Fig. 12-3) is a natural barrier to invading microorganisms. In addition fatty acids secreted by the glands of the skin can inhibit the growth of microorganisms. If one swabs the surface of the skin or removes material from sebaceous glands, a number of microorganisms can be cultured, among them species of *Staphylococcus* and *Corynebacterium*. These resident microorganisms are not pathogenic unless the skin becomes abraded and the individual's natural immune mechanisms become depressed. Microorganisms not indigenous to the skin may enter through skin abrasions, or they may be transferred by the bite of animals or insects. The rabies virus, for example, can be transmitted to humans by the bite of dogs, carnivorous wild animals (especially skunks, raccoons, foxes, and coyotes), and bats. Other infectious agents, including the causal agents of plague, malaria, and Rocky Mountain spotted fever, are transmitted by insect bites.

Mucosal Surfaces

The mucosal surface is one of the first surfaces on which microorganisms make contact with the host. The gastrointestinal tract, respiratory tract, salivary and lacrimal glands, biliary system, and portions of the genitourinary tract all have mucosal surfaces. Many of these surfaces are in contact with the external environment. A variety of host defense mechanisms are available at the mucosal surface to prevent parasite colonization and prevent damage by microbial products such as toxins:

1. Immunological defenses. The most important

Fig. 12-3. Cross section of the skin demonstrating the barriers to infectious agents.



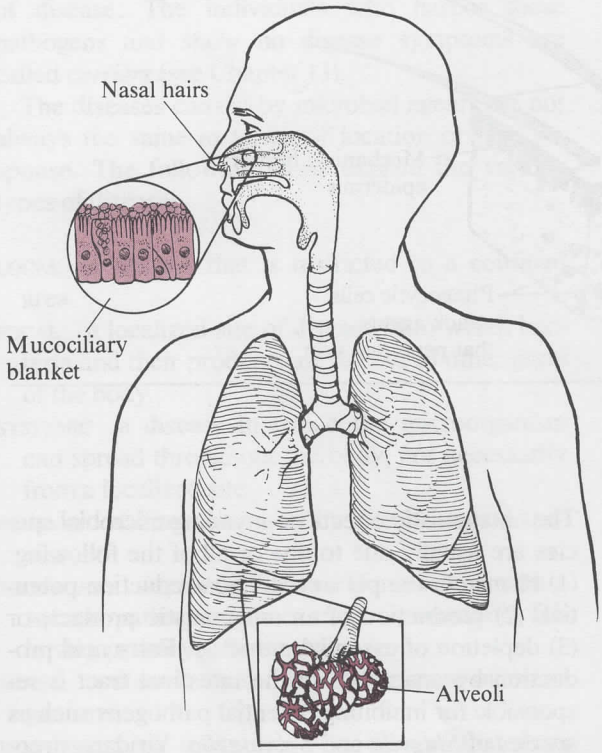
immunological defense at the mucosal surface is the immunoglobulin called secretory IgA (sIgA). Plasma cells located beneath the mucosal epithelia produce sIgA (see Chapter 15 for further details on this immunoglobulin). sIgA antibodies have been shown to prevent the binding of certain pathogenic microorganisms to the mucosal surface. Binding is an important property of most pathogenic microorganisms. Microbial species such as *Vibrio cholerae, Streptococcus mutans, Streptococcus salivarius*, and *Neisseria gonorrhoeae* will not bind to epithelial surfaces in the presence of sIgA. Local sIgA has also been shown to neutralize virus and thus play a role in protecting the host from reinfection by respiratory viruses. It should be pointed out that some pathogenic bacteria, such as species of *Neisseria, Hemophilus*, and *Streptococcus*, produce a protease that cleaves sIgA. Protease production may therefore be considered a virulence factor for some bacteria.

2. Microflora. The sites at which an invading microorganism may enter the host (skin, respiratory tract, digestive tract) are usually colonized by an indigenous population of microorganisms. The metabolic products of some commensal microorganisms can directly or indirectly affect the ability of noncommensal species to colonize a body site. The antagonistic effects on invading microbial species are usually due to one or all of the following: (1) change in the pH or oxidation-reduction potential, (2) production of an antagonistic product, or (3) depletion of essential nutrients. Fatty acid production by anaerobes in the intestinal tract is responsible for inhibiting potential pathogens such as species of *Shigella* and *Salmonella*. Viridans streptococci indigenous to the pharynx inhibit the growth of pneumococci, while *Staphylococcus epidermidis* retards the colonization of *Staphylococcus aureus* in the nasal cavity. The mechanisms involved in these interactions are not known, and much of the evidence is circumstantial since only in vitro experiments have been performed.

3. Mucins. Mucins are hydrophilic glycoproteins that form a film in the intestinal tract and a partial coat in the respiratory tract. They lubricate and waterproof the mucosal surface, but they have also been shown to prevent adherence of some microorganisms as well as their toxic products. Microorganisms bound by mucins may be removed via ciliary action or peristalsis from their initial site of infection.

4. Lysozyme, lactoferrin, and peroxidase. Lysozyme, lactoferrin, and peroxidase are three substances found on the mucosal surface that aid in de-

Fig. 12-4. Diagram of the respiratory tract showing the barriers to infectious agents. Alveoli contain phagocytic cells that engulf parasites.



fense against microbial invasion. Lysozyme is found in most normal secretions, including tears, nasal secretions, breast milk, and genital fluids. Its activity on the cell wall was discussed in Chapter 4. Lactoferrin is an iron-binding protein whose importance is discussed later in this chapter. Peroxidase is found in saliva and, in combination with thiocyanate and hydrogen peroxide, is active against several bacteria, fungi, and viruses (see Chapter 14).

Respiratory Tract

Inhaled air contains suspended particles of various sizes. The inhaled particles may include dust; epithelial cells shed from the skin, which may contain microorganisms; freely suspended molds or bacterial spores; and suspended aerosols from sneezing and coughing, which may also contain microorganisms. Particles that are 10 μm in diameter or more seldom reach the lungs because of the mucociliary blanket that covers the respiratory tract (Fig. 12-4). Cilia lining the nasal cavity trap particles and sweep them to the throat, where they are swallowed. The lower part of the respiratory tract contains macrophages that aid in resistance. These cells of the immune system can ingest and digest microorganisms. When host defense mechanisms for the respiratory tract become defective, some microorganisms are equipped to adhere strongly to the respiratory epithelium (Fig. 12-5) and may be the cause of serious infection such as whooping cough and bacterial and viral pneumonias. Other agents, the causative agent of tuberculosis, for instance, can be ingested by macrophages but are still capable of infection because immune responses are defective (see Chapter 17).

Oropharynx

Microorganisms in the oral cavity are numerous. Gingival fluid has as many as 1×10^{10} microorganisms per milliliter. Saliva is responsible for flushing out microorganisms from the oral cavity, and when salivary flow is impeded, for example, by anesthesia, the numbers of microorganisms increase. Saliva also contains such microbial inhibitors as lysozyme and secretory antibodies.

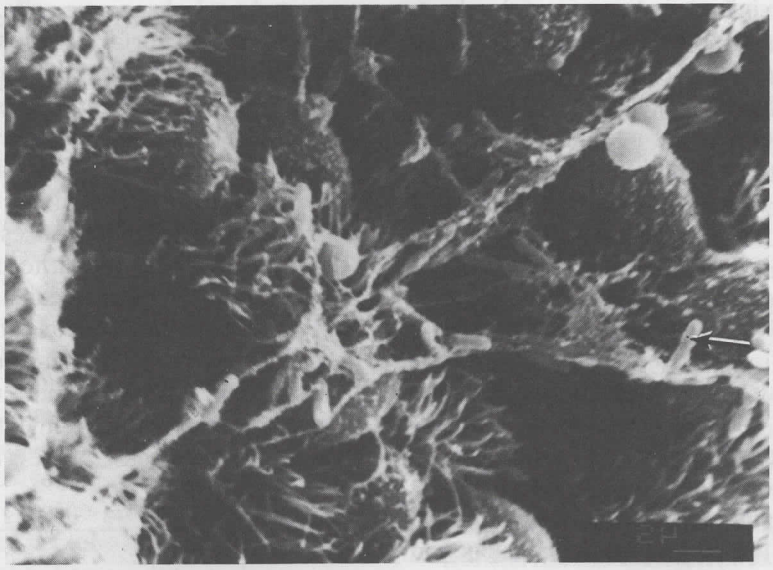
Intestinal Tract

Many microorganisms can enter the intestinal tract via the ingestion of food and drink. Those that are indigenous to the oral cavity are also swallowed. Organisms that survive the acid conditions of the stomach enter the intestinal tract and encounter acids, bile salts, and enzymes. Although the mucus lining the epithelial cells acts as a mechanical barrier to microbial colonization, some pathogenic species such as the causal agents of typhoid fever and plague resist these physiological barriers. Microorganisms indigenous to the intestinal tract, because of their metabolic activities, prevent colonization by pathogenic species, as is discussed later.

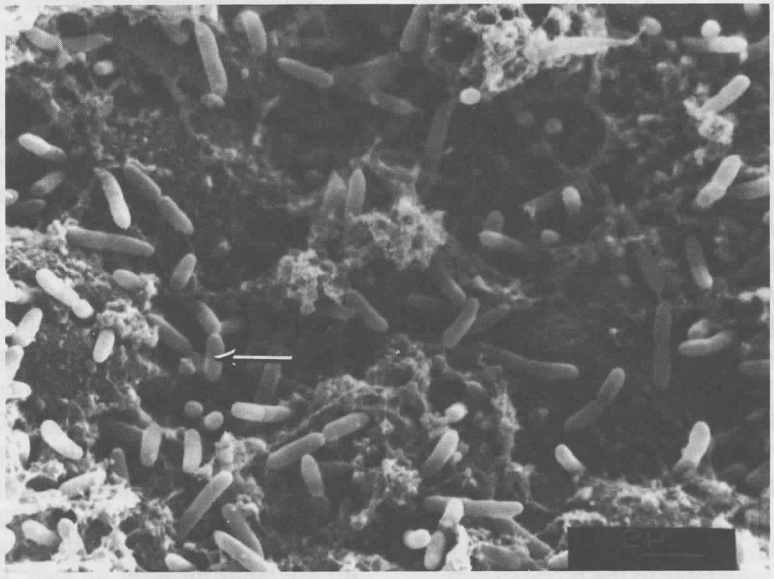
Urogenital Tract

Under normal conditions urine in the bladder is a

Fig. 12-5. Effect of an environmental factor on the adherence of microorganisms to respiratory epithelium. A. Normal tracheal cells in which a few rare bacteria (*arrow*) can be found adhering to cilia. B. Tracheal cells that have been injured with acid. Many bacilli (*arrow*) can be seen to adhere. (From R. Ramphal and M. Pyle, *Infect. Immun.* 41:345, 1983.)



A



B

sterile fluid. When it contains large numbers of microorganisms, serious infection is often indicated. Infections of the urogenital tract occur more frequently when structural abnormalities prevent the normal flow or flushing action of urine. Women are more prone to such infections because of the shortness of the urethra and its close proximity to the anus with its indigenous microflora. In women the normal acidity of the vagina is due to the presence of lactic acid, which inhibits many microbial species. Lactic acid is produced by bacterial degradation of glycogen, a major component of the vaginal epithelium.

EPITHELIAL AND SUBEPITHELIAL FACTORS

Once past the chemical and physical barriers at the sites of entry into the host, infecting microorganisms seek a site for multiplication and protection from the host's other defense mechanisms. The latter are directly or indirectly associated with the immune response of the host—interferon production and the production of antibodies and cells of the immune system that will immobilize or destroy the invading microbial species. The battle between microbe and host is influenced by both microbial and host factors but finally boils down to the resistance of the host.

Many microbial species colonizing the epithelial surface remain on the epithelium because they do not possess invasive factors for penetration into subepithelial tissue or because they are readily destroyed by antimicrobial factors in the subepithelial tissue of the host. Respiratory viruses such as those causing the common cold and viruses causing warts are examples of parasites that remain on the epithelium. It is not always necessary that the microorganism invade tissue to cause serious disease. The causal agent of diphtheria (*Corynebacterium diphtheriae*), for example, multiplies on the epithelial surface of the respiratory tract and produces a toxin that penetrates to the bloodstream.

Once the epithelial surface has been penetrated there are three host antimicrobial defense mechanisms: (1) tissue fluids, (2) the lymph system, and (3) phagocytic cells. Tissue fluids contain antimicrobial factors including antibodies and various serum factors such as properdin (see Chapter 15). A complex system of lymphatics, particularly near the skin and the intestinal wall, can carry microorganisms to lymph nodes where phagocytic cells are present. Local phagocytic cells are also present at the subepithelial surface at the time of penetration or when inflammatory conditions have taken place. Sometimes the microorganisms can evade these antimicrobial mechanisms. A few microorganisms, such as the agent of tuberculosis (*Mycobacterium tuberculosis*), are engulfed by the phagocyte but are resistant to phagocytic digestion. This provides the organism with a habitat for avoiding the host's remaining defense mechanisms. This is one reason why some diseases, such as tuberculosis and brucellosis, are usually of long duration and sometimes difficult to cure. A more detailed discussion of the cellular and fluid elements of the blood and their role in the disease process appears in Chapter 14.

RESISTANCE

As stated previously, the major factor that determines the outcome of microbial infection is the immunological state of the host. Today diseases such as measles are not nearly as devastating as they were several hundred years ago because people have developed a more benign relationship with the microorganisms causing them. Repeated infections in a community have resulted in the selection of more-resistant individuals. That this process has taken place is evident from studies of infectious diseases that have recently occurred in isolated communities for the first time. In Greenland, as well as among some jungle tribes of South America, first infections from smallpox have ravaged the populations. In developed countries, where smallpox has existed for a long time, a more benign relationship exists between host and pathogen. This long-term interaction has reduced the morbidity and mortality for other diseases as well. Resistance to infections is also influenced by species, race, age, sex, nutrition, and occupation. The influence of the immunological state of the host will be discussed in Section IV, Immunology.

Species

Some microbial agents can infect one species but not another. The rabies virus can cause disease in humans, dogs, and cats but has no effect on opossum or related animals. The reason for such species specificity is not known, but factors such as pH, temperature, and tissue chemistry and structure may contribute to these differences.

Race

Some races of humans are more susceptible to diseases than others. The fungal disease coccidioidomycosis is found more predominantly in dark-skinned individuals such as Hawaiians, Mexicans, and American Indians than in light-skinned persons. The reason for this is not known. A biochemical basis for racial immunity to malaria is known—in persons with sickle cell anemia, which affects blacks almost exclusively, there is resistance to malaria. The malaria parasite grows and develops in the erythrocyte. In persons with sickle cell, the conformation of the hemoglobin molecule has changed because of mutation, and parasite development in the erythrocyte is impeded.

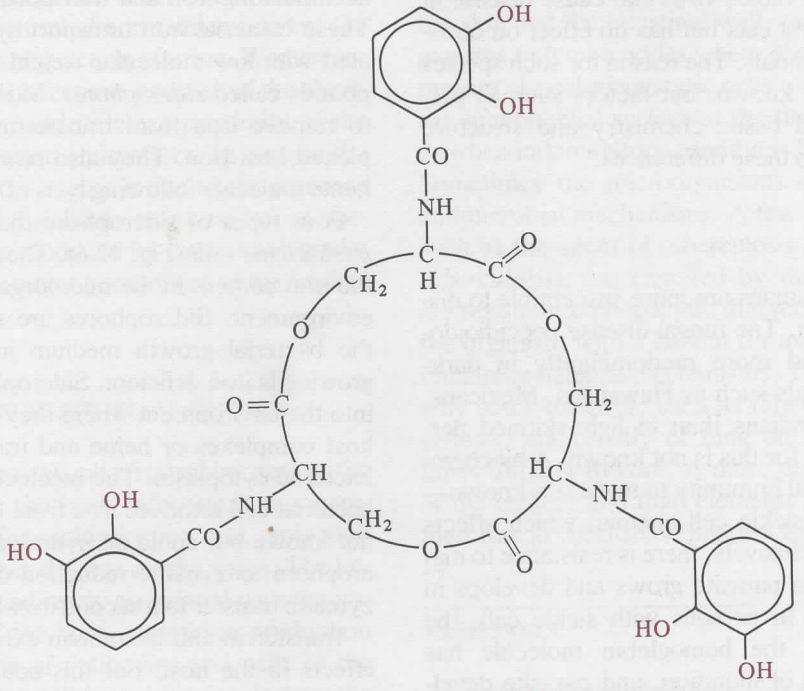
Nutrition

The nutrition of the individual may also contribute to the disease process. In general, one can say that if the state of health of the host is seriously impaired by inadequate nutrition infections are more likely to occur. Considerable evidence has shown that iron plays an important role in the host's susceptibility to disease. Iron is required by most living organisms. It is a component of cytochromes and is therefore involved in electron transport. Iron is found in high concentrations in the host (approximately 4.5 gm in an adult human), but most of this is unavailable to an infecting microorganism. Iron in host tissue is complexed with iron-binding glycoproteins such as transferrin or lactoferrin that are found in most body fluids. Host iron is also part of the heme molecule in red blood cells. Any free iron in the host is in an insoluble ferric form (Fe^{3+}), and this creates a condition in which the pathogen must compete with the host for available iron. Some microorganisms have evolved special mechanisms for accumulating iron and transporting it into the cell. These bacterial iron transport systems are associated with low molecular weight iron-binding compounds called *siderophores*. Siderophores are able to remove iron from transferrin and other complexed host iron. They also remove iron from the heme molecule following lysis of erythrocytes.

Four types of siderophores have been found in *Escherichia coli* (Fig. 12-6). They are regulated by the iron content in the microorganism's immediate environment. Siderophores are synthesized when the bacterial growth medium in which they are growing is iron deficient. Siderophores are released into the environment where they remove iron from host complexes or heme and transport it into the bacterial cytoplasm. The mechanism by which the bacterial cell removes iron from the siderophore is not known but could be hydrolysis of the iron-siderophore, enzymatic reduction of iron, or nonenzymatic transfer to a second iron-binding molecule.

Transferrin and lactoferrin exhibit bacteriostatic effects in the host, but this activity is lost when these molecules become saturated with iron. Lactoferrin is present in high concentrations in human milk and is probably responsible, along with antibody, for inhibiting microorganisms such as *E. coli* that cause gastroenteritis in infants. Several studies have shown that breast-fed babies are more protected against enteritis caused by *E. coli* and other microorganisms than are non-breast-fed babies. Breast milk has occasionally been used to stop outbreaks of enteritis when all other methods of treatment have failed. Microorganisms whose virulence has been associated with excess iron include *Yersenia pestis* (plague), *Neisseria meningitidis* (meningitis), and *N. gonorrhoeae* (gonorrhea). Experiments using chick embryo have demonstrated that bacterial species, such as *Hemophilus influenzae* type B, that cause disseminated disease are able to acquire iron whether the serum is saturated or relatively unsaturated with iron. Bacterial species that have low levels of iron and cause localized disease, for example, *V. cholerae*, the agent of a diarrheal disease, have been shown to have inefficient mechanisms for acquiring iron.

Fig. 12-6. Structure of one type of siderophore. Sites of iron binding are in color.



Occupation

Occupation is an important factor in susceptibility to infection. One would expect more infections of tularemia (rabbit fever) and brucellosis in persons who are exposed to animals and animal hides—hunters, stockyard workers, veterinarians. Hepatitis, which can be acquired through infected blood or blood products, is more prevalent among surgeons, nurses, and others who work in hospitals than in the general population.

Underlying Conditions

Underlying conditions or diseases are major predisposing factors to infection. Individuals who produce no immunoglobulins or abnormal ones are susceptible to repeated microbial infections. Diabetics whose disease is not under control by insulin or diet are particularly prone to tuberculosis and staphylococcal infections. Many viral infections can reduce the patient's resistance to the point at which secondary bacterial infections are common. For example, viral influenza is sometimes followed by a staphylococcal infection that may be worse than the original viral infection.

Numerous conditions that predispose to disease can be induced in the host. For instance, corticosteroid treatment and ionizing radiations used in cancer therapy reduce antibody formation and interfere with the normal inflammatory response. Under these circumstances the patient is at the mercy of what are normally mild infectious agents such as the chickenpox virus and many gram-negative bacteria of the intestinal flora. The advent of transplants has necessitated the use of immunosuppressive drugs to prevent rejection of the foreign tissue or organ by the host. As a result of immuno-

Table 12-1. Microbial Products Associated with Invasiveness and Spread of Microorganisms in the Host

Invasive factor	Organism	Function
Coagulase	Staphylococci	Causes formation of fibrin, which may deposit on surface of microorganism and inhibit phagocytosis
Streptokinase (fibrinolysin)	Streptococci	Transforms plasminogen to plasmin, an active proteolytic enzyme that digests fibrin; importance in disease not totally understood
Hyaluronidase	Streptococci, staphylococci, and clostridia	Splits hyaluronic acid, a component of connective tissue
Hemolysins	Many bacteria, especially streptococci, staphylococci, and clostridia	Lyse red blood cells
Lecithinase	Clostridia	Splits lecithin, a component of cytoplasmic membranes
Collagenase	Clostridia	Digests collagen, a component of connective tissue
Extracellular polymeric substances (capsules and slime layers)	Several bacteria, including pseudomonads, staphylococci, streptococci, and others	Inhibits phagocytosis and in some instances may be important in adhering to host tissue

suppressive therapy patients often survive the transplant only to succumb to microbial infection. The patient with heart disease is also susceptible to infections of the heart tissue. Some streptococci in the oral cavity, for example, may be displaced into the bloodstream during oral surgery. In the healthy person these microorganisms are rapidly cleared from the circulation. In the patient with heart disease they may lodge in the heart tissue and set up foci of infection.

MICROBIAL FACTORS CONTRIBUTING TO INFECTIOUS DISEASE

INVASIVENESS OF BACTERIA

Invasiveness refers to the ability of microorganisms to penetrate tissue. Invasiveness is not always associated with virulence or vice versa. For example, *Clostridium tetani*, the causative agent of tetanus, is not an invasive microorganism but is extremely virulent once it has infected the host because of the release of a potent toxin. Many viruses are capable of invading tissue but are not pathogenic to humans.

Some virulent microorganisms such as *C. diphtheriae* are restricted to the type of tissue that is suitable for their multiplication. *C. diphtheriae* organisms invade only the superficial cells of the upper respiratory tract. Some fungi, however, can multiply and invade a variety of host tissue and organs. The reason for these differences in tissue affinity has not been clearly defined. Many may be due to the chemical environment of the host tissue. The fact that *Proteus mirabilis*, for example, produces more severe and persistent infections in the kidney than elsewhere is perhaps related to its production of urease, an enzyme that splits urea into carbon dioxide and ammonia, thereby promoting the growth of this microorganism. *Brucella abortus*, which causes abortion in cattle, localizes in the placenta, the only area containing erythritol, a polyhydric alcohol that *B. abortus* can rapidly metabolize.

The invasiveness of bacteria is associated with their ability to produce certain extracellular enzymes (Table 12-1). These enzymes are discussed at more length in connection with the bacterial diseases with which they are associated. A great deal of controversy has been raised concerning the role, if any, of these enzymes in the pathogenic process. For example, the virulence of *S. aureus* was at one time attributed to the ability of the microbe to produce the extracellular enzyme coagulase. Coagulase has been shown in vitro to cause the deposition of fibrin around the bacterial cell, presumably protecting it from antibacterial substances in the blood. Virulent strains of *S. aureus* have been found, however, that produce no coagulase. Although no single enzyme has been unequivocally associated with virulence, it is certain that enzymes play some role in the pathogenic process.

TOXINS

Two types of toxins are produced by bacteria: exotoxins and endotoxins. The major distinctions between the two types are outlined in Table 12-2.

Table 12-2. Characteristics Differentiating Exotoxins from Endotoxins

Exotoxins	Endotoxins
Most are polypeptides with molecular weights between 1×10^4 and 9×10^5 daltons	Low molecular weight component of lipopolysaccharide complex; lipid A is toxic component
Excreted by living cells	Part of the cell wall of gram-negative bacteria and can be released when the cell lyses or during vegetative growth
Relatively unstable to temperatures above 60°C	Relatively stable to temperatures above 60°C for several hours with no loss of activity
Antigenic (stimulates formation of antibodies)	Lipid A is not antigenic
Can be converted to a toxoid	Cannot be converted to a toxoid
Does not produce fever in the host	Produces fever in the host
Very toxic in microgram quantities to laboratory animals	Weakly toxic; hundreds of microgram quantities required to be lethal for animals

Exotoxins

Exotoxins are heat-labile proteins released by the bacterial cell into the surrounding environment. Most exotoxins are produced by gram-positive bacteria. They include some of the most potent poisons known, such as the diphtheria, tetanus, and botulism toxins. Exotoxins whose site of action is the intestinal tract are called *enterotoxins*.

Most exotoxins are highly antigenic; that is, they can stimulate the formation of antibodies (*antitoxins*) when injected into appropriate hosts. For this reason exotoxins can be used to induce active immunity against toxin-caused diseases such as diphtheria and tetanus. However, because many toxins are potent and lethal poisons, they cannot be injected into the human body without some modification of the toxin molecule. Exotoxins can be modified chemically by phenol, formaldehyde, betapropiolactone, and various acids. In its modified form the toxin is called a *toxoid*. When injected into the body, toxoids have the ability to stimulate the formation of antibodies that neutralize the specific exotoxins from which they were derived, and toxoids are not toxic to the host. Toxoids or inactivated virulent microorganisms used in immunization are referred to as *vaccines*. (The term *vaccine* was previously reserved for killed or attenuated microorganisms and not their products. A more complete definition of *vaccine* must now include specific microbial components. For example, a surface antigenic component on the hepatitis virus is now used as a vaccine.) The major exotoxins produced by pathogenic bacteria and their activities are listed in Table 12-3.

Most exotoxins are composed of two subunits; one is nontoxic but binds to specific cell surface receptors and facilitates the entry of the second subunit, which is the toxic component. The exotoxins

Table 12-3. Important Exotoxins Associated with Disease in Humans

Toxin or disease	Microorganism	Mechanism of action
Exotoxin A	*Pseudomonas aeruginosa*	Inhibits peptide chain elongation
Enterotoxin	*Bacillus cereus*	Similar to cholera toxin
Enterotoxin (traveler's diarrhea, infant diarrhea)	*Escherichia coli*	Same as cholera toxin
Scalded skin syndrome	*Staphylococcus aureus*	Separation of the epidermis
Erythrogenic toxin (scarlet fever)	*Streptococcus pyogenes*	Produced only by strains of streptococci carrying a bacteriophage that is nonvirulent; toxin causes a rash
Enterotoxin (food poisoning)	*Staphylococcus aureus*	Neurotoxin that affects motility of the gut
Diphtheria toxin	*Corynebacterium diphtheriae*	Inhibits peptide chain elongation during protein synthesis
Cholera enterotoxin	*Vibrio cholerae*	Stimulation of adenyl cyclase and increase in cAMP, leading to fluid and electrolyte loss from the gut
Tetanus or lockjaw	*Clostridium tetani*	Neurotoxin blocks function of inhibitory synapses in the spinal cord, thus causing muscular spasms
Botulism (types A, B, and E toxins)	*Clostridium botulinum*	Neurotoxin that blocks release of acetylcholine or blocks its production at synapses or neuromuscular junctions
Enterotoxin	*Clostridium perfringens*	Causes hypersecretion of electrolytes and water in the intestine
Alpha toxin (gas gangrene)	*Clostridium perfringens*	Splits lecithin, a component of cytoplasmic membranes

cAMP = adenosine 3′:5′-cyclic monophosphate.

of enteropathogenic *E. coli* and *V. cholerae* exert their activity in the intestinal lumen. Both of these species cause diarrhea via the action of toxin on the enzyme adenyl cyclase in the mammalian cytoplasmic membrane. The enzyme catalyzes the following reaction.

$$\text{Adenosine triphosphate (ATP)} \xrightarrow[\text{cyclase}]{\text{adenyl}} \text{adenosine 3′:5′ cyclic monophosphate (cAMP)}$$

The increase in cAMP causes a change in the electrical potential of the mammalian cytoplasmic membrane, and this results in a leakage of fluids and electrolytes. *C. diphtheriae* and *Pseudomonas aeruginosa* also produce exotoxins whose mechanism of action is similar. They inhibit protein synthesis by preventing the transfer of amino acids to the growing polypeptide chain. This is accomplished by inactivation of the translocating enzyme. The diphtheria toxin is synthesized as a single polypeptide that is fragmented by a bacterial protease. Only the fragmented polypeptide shows toxic activity. Although the mechanism of action of *C. diphtheriae* and *P. aeruginosa* toxin are alike, they are specific to different tissues and are also structurally different.

Endotoxins

As discussed in Chapter 4, an endotoxin is a complex found in the gram-negative cell wall. The complex is made up of a core of oligosaccharides to which a lipid, called lipid A, is bound. The toxic component of the endotoxin complex is believed to be lipid A. Also bound to the core are the O-specific side chains, polymers of oligosaccharide units, that carry the antigenic determinants responsible for the serological specificity of the bacterium. Endotoxin is released usually when the cell is lysed, but can also be released during vegetative growth.

Endotoxin has been isolated from gram-negative bacteria, and its biological properties have been determined through studies of a number of susceptible animals. The effects of endotoxin on the cardiovascular, respiratory, and blood-clotting mechanisms have been known to lead to septic shock and death. Evidence of the role endotoxin plays in gram-negative septicemia in humans is circumstantial even though people are very sensitive to intravenous injections of purified endotoxin. What happens when endotoxin is released during infection by gram-negative bacteria is not completely understood. The clinical features of fever, intravascular coagulation, and depression of the reticuloendothelial system that appear in animals injected with endotoxin are sometimes duplicated in human gram-negative bacteremia. These features are as follows:

1. Fever. Endotoxin has the ability to induce fever in susceptible animals. Endotoxin causes macrophages and other cells to release endogenous pyrogens. The pyrogen is a protein and acts on the temperature-regulating center, that is, the anterior hypothalamus. Continuous infusions of endotoxin in animals can lead to a loss of response to the pyrogenic effect, and it has been suggested that the continued fever in typhoid fever, a gram-negative infection, may be due not to endotoxin but to other factors.
2. Activation of the coagulation mechanism. Intravascular coagulation is one of the manifestations of gram-negative bacteremia, although it also occurs in other types of infections. Endotoxin activates components of the clotting mechanism and thus leads to the formation of fibrin. If fibrin becomes trapped in the small blood vessels, intravascular coagulation and shock can follow.
3. Depression of the reticuloendothelial system. Macrophages make up the reticuloendothelial system, and endotoxin prevents the macrophages from clearing the fibrin polymers that become trapped in the small blood vessels.
4. Vascular collapse. In animals endotoxin is known to cause the release of biologically active substances from the cells of the immune system. Some of these active substances affect the circulatory system; they can cause increased vascular permeability and dilatation. The circulatory changes lead to impaired blood flow to vital organs—kidneys, liver, brain, and lung—and ultimately to shock and possibly death. These vascular reactions are also seen in gram-negative bacteremia in humans.

Among the major effects attributed to endotoxin are

1. Pyrogenicity (ability to induce fever)
2. Lethal toxicity for mice
3. Leukocytosis
4. Complement (a series of proteins found in serum that is associated with immune reactions) activation
5. Depression of blood pressure
6. Induction of nonspecific resistance to infection
7. Induction of tolerance to endotoxin
8. Induction of early refractory state to endotoxin pyrogenicity
9. Macrophage activation
10. Induction of interferon production

Whether endotoxin released during gram-negative bacteremia is responsible for these effects has only been surmised. It is currently believed that interaction of endotoxin with the macrophage may be the primary event in initiating all of the biological effects of endotoxin. Several in vitro and in vivo studies with endotoxin have demonstrated that these bi-

ological effects can be produced by endotoxin-stimulated macrophages.

There are many gaps in our knowledge concerning the actual effect of endotoxin in gram-negative bacteremia. It has been shown that the core portion of the endotoxin injected into animals protected them from infection by a wide variety of heterologous gram-negative species. This is an important finding when one considers the following statistics: In those patients in whom bacteria have invaded the bloodstream, shock occurs in about 25 percent, and about 45 percent of these die of gram-negative infection. Because gram-negative species are the most commonly isolated species from hospital-associated infections, a vaccine would be an important breakthrough in prevention.

ANTIPHAGOCYTIC FACTORS

Several species of bacteria owe their virulence to their ability to evade phagocytosis or, if the organism is phagocytized, to prevent phagocytic digestion. Capsule formation by some species of bacteria, such as *Streptococcus pneumoniae, Klebsiella pneumoniae, Bacillus anthracis*, and *Yersinia pestis*, is sufficient to inhibit phagocytosis (Fig. 12-7). Capsule interferes with the attachment of phagocytic cells, such as neutrophils, to the microbial surface. The presence of a capsule is not always sufficient to prevent phagocytosis, and many encapsulated bacterial species are rapidly ingested and killed by phagocytic cells.

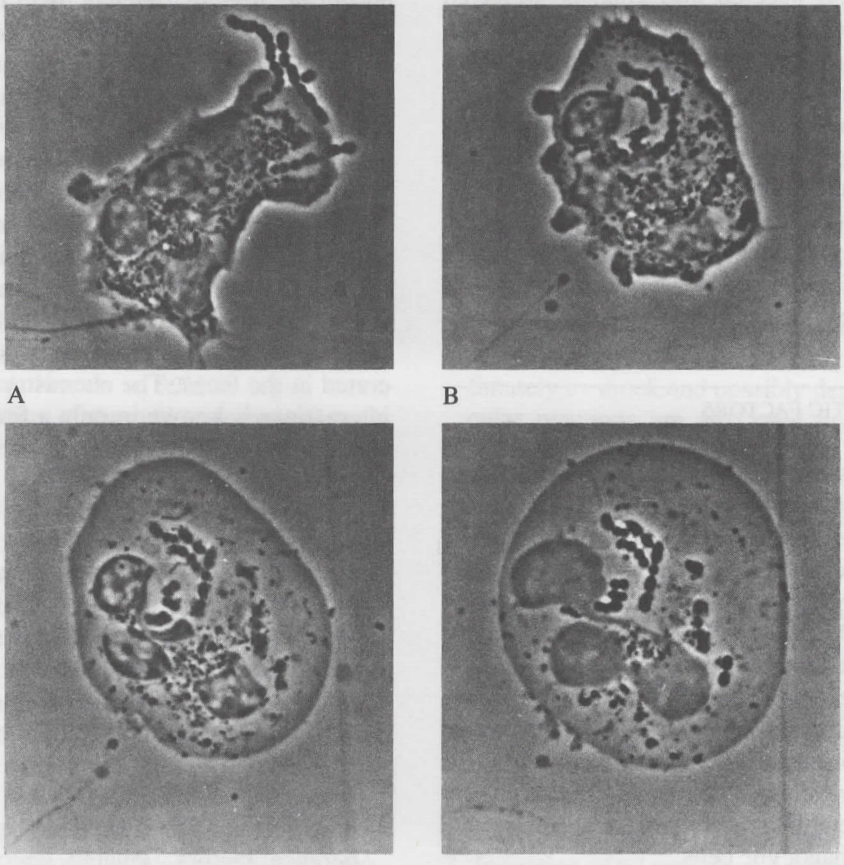
S. aureus demonstrates resistance to phagocytosis because of a surface antigen as well as a cell wall component called protein A. Some microbial species such as *Mycoplasma pneumoniae* and *N. gonorrhoeae* are bound to the phagocyte but not ingested. Organisms such as species of *Salmonella, B. abortus*, and *S. aureus* survive within the phagocytic cell by preventing the release of antimicrobial factors (see Chapter 14).

BACTERIAL ADHERENCE

Bacterial adherence is of obvious importance for those microbial species that are commensals in the host. These species have surface components called *adhesins* that enable them to bind to mammalian cells and to form microcolonies. Some potential pathogens must also be equipped with adhesins in order to cause disease in the host. In addition, the host tissue must have receptors that are specific for the particular microbial adhesin. If there is no mechanism for attachment, the microorganisms will be largely removed from potential sites of infection. For example, microorganisms in the upper respiratory tract may be swallowed and eventually destroyed by stomach acids; those in the intestine may be removed by peristalsis and excreted in the feces. The chemistry of host-parasite interactions is known in only a few instances. Examples of these interactions are as follows:

1. *N. gonorrhoeae* shows an attachment specificity for epithelial cells in the genital tract and buccal cavity. Current studies indicate that adherence may require more than one step and involve more than one adhesin. The first step is attachment of the microbial cell to the microvilli on the surface of nonciliated cells (Fig. 12-8). This is followed by attachment to the surface of the nonciliated cells. Of the known gonococcal surface components only pili and a protein called protein II have been examined extensively as adherence factors. Piliated gonococci attach in greater numbers to human fallopian tube mucosa than do nonpiliated gonococci. However, it has also been demonstrated that nonpiliated gonococci attach more readily to the same tissue than do commensal species. This indicates that factors other than pili are probably involved in this selective attachment process. Protein II is an outer membrane protein of the gram-negative gonococcus that does not appear to play a direct role in its initial attachment to the fallopian tube mucosa. Protein II is believed to promote either the attachment of gonococci to nonciliated cells of the fallopian tube or the aggregation of gono-

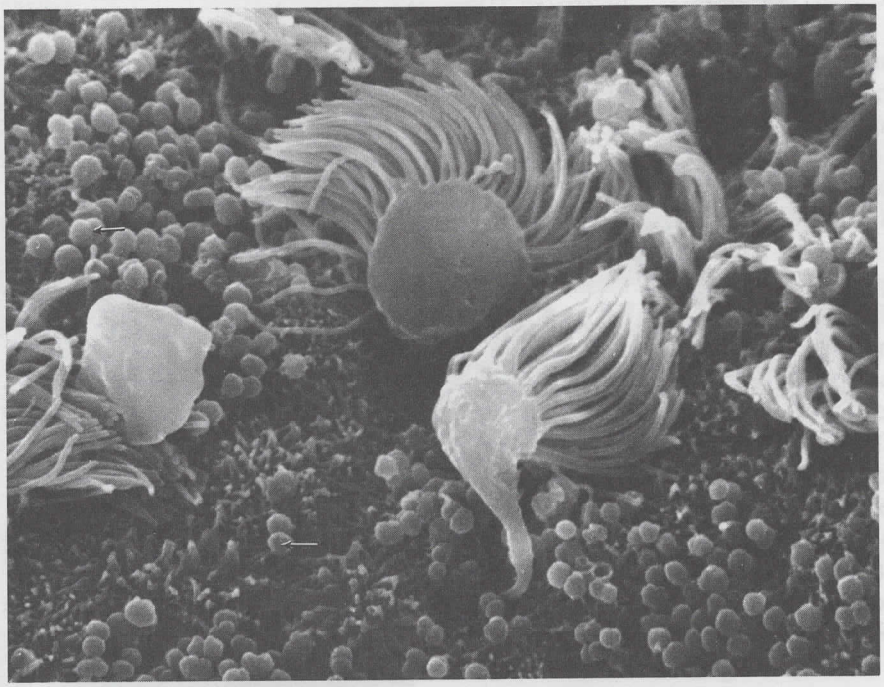
Fig. 12-7. Phase-contrast micrograph demonstrating leukotoxic action of streptococci on human neutrophils. A. Neutrophil is seen to be engulfing three streptococcal chains. B. Neutrophil has ceased moving and has begun to round up. Cytoplasmic blebs are seen to emanate from its surface. C. Neutrophil is swollen and immobile. D. Most internal structures of neutrophil have disappeared, and nucleus appears swollen. (From G. W. Sullivan and G. L. Mandell, *Infect. Immun.* 30:272, 1980.)



cocci. Both pili and protein II appear in different molecular forms among the gonococci of a given strain. When gonococci are cultured in vitro these molecular forms change at a high frequency. It is suggested that, in the host, gonococci may modify these forms to cope with different anatomical sites and the accompanying change in host receptors.

2. *Streptococcus pyogenes* adheres to epithelial cells of the skin and nasopharynx of the human host. One of the surface components, lipoteichoic acid (LTA), plays an important role in attachment. Two other surface components, the M protein and hyaluronic acid capsule, prevent recognition by phagocytes. LTA, which consists of a backbone of polyglycerophosphate attached to a glycolipid, is secreted through the cytoplasmic membrane. Some of the LTA molecules

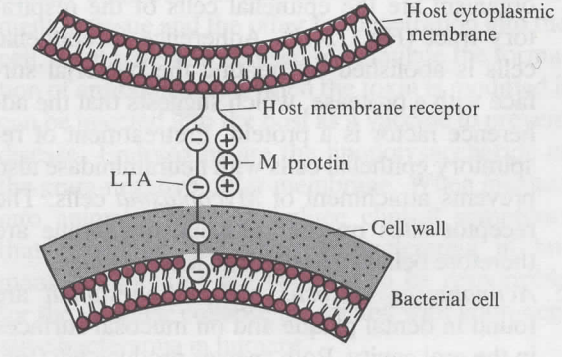
Fig. 12-8. Scanning electron micrograph (×3126 before 50% reduction) of *Neisseria gonorrhoeae* attached to human fallopian tube organ cultures. Note intact ciliated cells (*far right and top*) and two sloughing intact ciliated cells with full complement of cilia (*center*). Gonococci (*arrow*) are attached to microvilli of nonciliated cells. (From Z. A. McGee, et al., *J. Infect. Dis.* 143:413, 1981.)



join with the M protein, which leaves the lipid ends exposed near the surface of the bacterium (Fig. 12-9).

3. Some strains of *E. coli* cause enteric disease. These are divided into two types: those that are invasive and cause a dysentery-like syndrome and those that are not invasive and cause diarrhea. Colonization of the intestinal epithelium by *E. coli* is required to prevent dislodgement due to the flushing action associated with peristalsis of the gut. A surface antigen called the K antigen (*K* stands for the German word for capsule, *kapsel*) is an important adhesin in various strains of *E. coli* infectious for animals. Similar adhesins present in human strains of *E. coli* resemble protein pili-like appendages (Fig. 12-10). The majority of pathogenic strains produce type I pili whose adhesive properties are inhibited by

Fig. 12-9. Lipoteichoic acid (*LTA*) attachment process. LTA associated with the cytoplasmic membrane of the bacterial cell is negatively charged. Positively charged M protein binds to LTA at the surface, leaving exposed lipid ends that bind to receptors on the host cell.



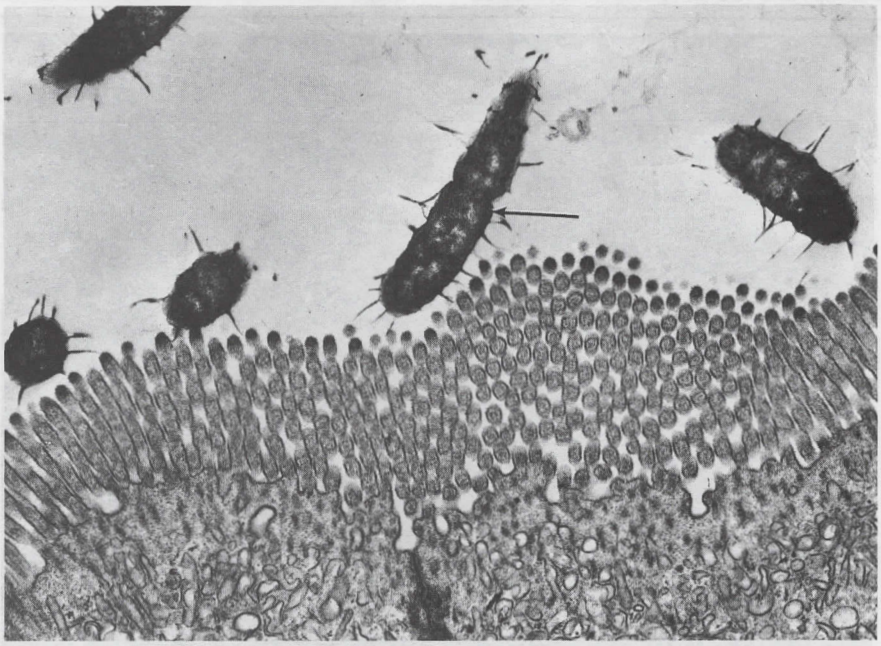


Fig. 12-10. Electron micrograph demonstrating attachment of *Escherichia coli* (*arrow*) to the intestinal epithelium. The pili can be seen to project from the surface of the bacterial cells and are in contact with host cell surface. (From H. W. Moon, B. Nagy, and R. E. Isaacson, *J. Infect. Dis.* 136 (Suppl.):124, 1977.)

D-mannose. A small number of *E. coli* produce a specific adhesin that is associated with diarrhea in adults and children. These adhesins are referred to as *colonization factor antigens CFA/I* and *CFA/II*.

4. *Mycoplasma pneumoniae* is a cause of one type of bacterial pneumonia. The target cells of this organism are the epithelial cells of the respiratory tract (Fig. 12-11). Adherence to epithelial cells is abolished by treating the bacterial surface with a protease, which suggests that the adherence factor is a protein. Pretreatment of respiratory epithelial cells with neuraminidase also prevents attachment of *Mycoplasma* cells. The receptor sites on the epithelial membrane are therefore believed to be sialic acid residues.
5. *Actinomyces viscosus* and *A. naeslundii* are found in dental plaque and on mucosal surfaces in the oral cavity. Both species produce pili (Fig. 12-12) that promote their adherence to host tissue. Other oral microorganisms such as *Streptococcus mutans* produce a slime layer (plaque) that favors attachment to the tooth surface; these are discussed in Chapters 19 and 37.

SUMMARY

Microorganisms that thrive in or on host tissue are called parasites. Some of them remain as part of the normal microbial flora of the host and are referred to as commensals. Others, however, elicit a host response and cause infectious disease. The site harboring the infectious agent is controlled by both host and microbial factors. Some infectious agents remain in the extracellular environment of the host, while others prefer or require an intracell-

Fig. 12-11. Scanning electron micrograph (×8000 before 33% reduction) of *Mycoplasma pneumoniae* in which the microorganisms (*M*) are clustered and attached to ciliated epithelial cells. Filamentous *Mycoplasma* cells (*arrows*) and cilia (*C*) can also be observed. (Courtesy Michael G. Gabridge.)

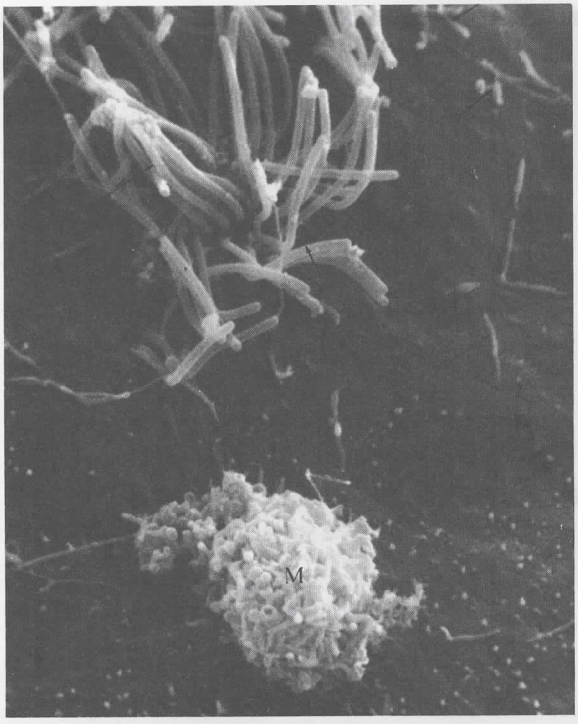
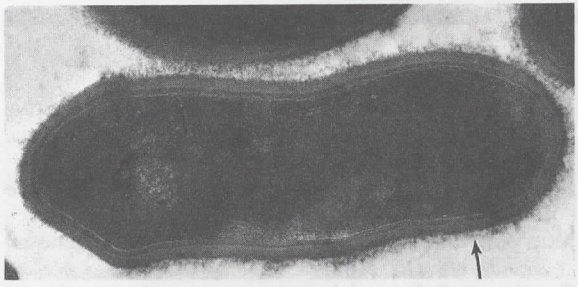


Fig. 12-12. Electron micrograph (×68,000 before 30% reduction) of *Actinomyces naeslundii* showing fuzzy pili-like material (*arrow*). (From C. H. Lai, *Infect. Immun.* 25:1016, 1979.)



ular habitat. Microorganisms that inhabit the host and form colonies but do not elicit an immune response are called colonizers. Microorganisms that elicit an immune response but cause no overt symptoms are said to cause an infection; those that do elicit symptoms are said to cause disease.

The ability to cause disease, that is, the pathogenicity of the organism, can be expressed quantitatively as a virulence factor. This virulence is usually related to the ability of the pathogen to invade tissue, to produce toxins, or both.

Host factors that inhibit microbial invasion include the physical and physiological barriers at the portals of entry such as the respiratory tract and intestinal tract. Host defenses that are part of the epithelial and subepithelial surface include chemical tissue factors, the lymph system, and phagocytic cells. In addition, the host's microflora also influences the site of colonization by the invading microorganisms. Other host resistance factors include species, race, age, nutrition, occupation, and underlying disease. Considerable experimental evidence indicates that iron plays a very important role in the infectious disease process. Microorganisms must extract iron from the host to survive. This extraction is accomplished through the production of iron-binding compounds called siderophores.

Many microbial factors influence the outcome of the host-parasite relationship. Some bacteria can invade or spread through tissue because of the production of certain enzymes. Other bacteria cause disease by producing toxins. Toxins are of two types, exotoxins and endotoxins. Exotoxins are usually composed of two subunits: one for attachment to tissue and the other for penetration into the cell. Exotoxins can be used to stimulate the formation of antibodies, and when the toxin is modified it can be injected into the host as a vaccine to prevent disease. Endotoxins are the lipopolysaccharide of the gram-negative outer membrane. When injected into animals they can induce clinical symptoms that resemble gram-negative bacteremia in humans. Endotoxins are believed to be responsible for the vascular collapse associated with gram-negative bacteremia in humans.

Some bacteria are capable of resisting one of the host's major defense mechanisms—phagocytosis and phagocytic digestion. This resistance is associated with various surface components such as capsules and certain surface proteins. One of the most important factors that enables microorganisms to carry out their activities in the disease process are adhesins. Adhesins are surface-associated components, such as pili, that enable the microorganism to remain attached to host tissue so that microcolonies can be produced and microbial factors associated with disease can be released.

QUESTIONS FOR STUDY

1. Which areas of the body are inhabited by microorganisms? Which areas are essentially sterile?
2. Is there any difference between an indigenous microbial species and a commensal?
3. Why do some microorganisms invade red blood cells, while other microorganisms invade white blood cells or macrophages?
4. What is the distinction between pathogenicity and virulence?
5. What is the distinction between infection and disease?
6. What is meant by a systemic disease? What is the opposite of systemic disease called?
7. What factors determine whether an inhaled particle containing microorganisms or microbial spores will reach the lower respiratory tract?
8. What host defense mechanisms are available at the epithelial and subepithelial surface?
9. How could ingested microorganisms reach the intestine without being destroyed by the acid conditions of the stomach?
10. Why are women more prone than men to urogenital infectious disease?
11. In general terms what is the mechanism by which exotoxins are taken up by susceptible host cells?
12. Name some infectious diseases in which an exotoxin is involved.
13. Give some examples in which a host genetic factor is associated with resistance to disease.
14. What are bacterial siderophores? Why are they produced by infectious microorganisms?
15. List some microbial products that enable the organism to spread and to invade host tissue.
16. What are the principal distinctions between an exotoxin and an endotoxin?
17. Name some important microbial adhesins and the infectious agents with which they are associated.

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13. EPIDEMIOLOGY

OBJECTIVES

To describe the various stages and symptoms associated with acute disease

To understand the mechanisms that enable microorganisms to persist in the host

To differentiate between reservoirs and sources of disease

To list those diseases that are transmitted from animals to humans

To list those diseases that are transmitted to humans from contaminated water or food

To describe how microorganisms can be transmitted by air

To briefly explain the laboratory techniques used to identify the source of a disease

To define: chronic disease, subclinical infections, pyrogen, carrier, zoonoses, vector, droplet nuclei, fomite, epidemic, endemic, herd immunity, morbidity, index case, biotype, serotype

Clinical Stages of Disease in the Host
Acute vs. Chronic Disease
Acute Disease
Incubation Period
Prodromal Period
Acute Period
Convalescent Period
Chronic Disease and Persistence
Reservoirs and Sources of Disease
Animate Reservoirs
Human
Animal
Insect
Inanimate Reservoirs
Transmission of Infectious Microorganisms
Contact
Direct
Indirect
Water
Food
Fomites
Air
Horizontal vs. Vertical Transmission
Vertical Transmission
Patterns of Disease in the Community
Endemic, Epidemic, and Sporadic Disease
Incidence and Prevalence
Herd Immunity
Descriptive Epidemiology
Host Factors
Place
Time
Laboratory Techniques in Epidemiology
Biotyping
Serotyping
Antibiograms
Phage typing
Bacteriocin Typing
Other Methods

In the previous chapter we discussed the one-on-one relationship between host and parasite. Epidemiology (from the Greek *epidemios,* which means "among the people") is concerned with disease as it applies to populations of individuals. The science of epidemiology may be involved with infectious as well as noninfectious disease. The role of the epidemiologist is to gather statistics that will indicate the prevalence or frequency of disease. Our discussion of epidemiology will be directed at how infectious diseases are spread, how they are controlled, and how they may be identified, as well as their frequency and prevalence in a population.

CLINICAL STAGES OF DISEASE IN THE HOST

To understand the various aspects of disease in a population we should first have some knowledge of the clinical stages of infectious disease in the individual.

ACUTE VS. CHRONIC DISEASE

Individuals infected by a pathogenic microorganism will respond to it in various ways. Sometimes the host's immune system will inhibit the pathogen before it can elicit symptoms or the symptoms will be so slight as to be almost imperceptible. Such infections are called *subclinical* or inapparent. When the pathogen overcomes the host's defense mechanisms, the manifestations of disease may ensue and proceed in an acute or chronic manner.

Acute Disease

Acute disease is characterized by symptoms that usually appear quickly, become very intense, and then subside when the host's immune system has overwhelmed the pathogen or its toxic products.

Acute disease is the type of disease with which most of us are acquainted, for example, the childhood diseases such as measles and chickenpox and the various types of influenza. One can always determine the approximate length of time it will take to recover from such diseases. Acute disease can be divided into various stages: *incubation, prodromal, acute*, and *convalescent*.

INCUBATION PERIOD. The incubation period is that period of time from the moment the infectious agent enters the host until the first symptoms of disease appear. This period may be as short as a few hours or as long as several years. The time interval is influenced by how fast the infectious agent can multiply and how quickly the microorganisms or the microbial products affect host tissue. Food poisoning caused by the ingestion of preformed toxin in contaminated food often produces symptoms within 8–24 hours. Other diseases, in which the infectious agent is taken into the body, may have incubation periods of one to several weeks. Many of the fungal pathogens, for example, have long incubation periods in the host. One must remember that the incubation period is greatly influenced by the virulence of the microorganism, the antigenic potential of the microorganism or its products, and the immunological state of the host. Measles affecting a normal healthy child in the community may have an incubation period of 7–10 days, but the same virus infecting a hospitalized patient could have an incubation period of 1–2 days. The average incubation period for most diseases is between 10 and 21 days.

PRODROMAL PERIOD. The term *prodromal* is derived from the Greek word that means "running before." In terms of the infectious disease cycle *prodromal* refers to the warning symptoms that indicate the onset of disease. This is the period in which the individual characterizes his or her general state of health as "not feeling well." The technical term for this symptom is *malaise*. It may include such symptoms as headache, upset stomach, or slight fever. The infectious agent during the prodromal period has multiplied only to the extent that its products have induced a slight response by the host.

ACUTE PERIOD. The acute period is the stage in which the symptoms of disease are at their peak. During this period the infectious microorganisms have reached a population level that induces the host to respond with immunological intensity. The intensity of this immunological response as well as the intensity of the symptoms are related to the number of host-parasite encounters. If the encounter is the first of its kind then the symptoms of disease are usually very intense and the maximum immunological response takes longer to develop. A rapid immunological response and less intense symptoms will develop if the disease represents a repeat encounter between host and parasite.

The types of symptoms associated with the acute period vary from disease to disease, but some symptoms are more prevalent in most diseases than others. Fever is one of the most common symptoms of disease and is mediated by substances called *pyrogens*. Pyrogens are small proteins synthesized and secreted by special phagocytes in response to stimuli. The cells producing pyrogens include neutrophils, monocytes, tissue macrophages, and phagocytic cells of the reticuloendothelial system. The increase in temperature is often related to the number of microorganisms or the amount of their products that appear in the bloodstream. Undulating types of fevers represent bursts of organisms appearing in the bloodstream from localized sites of infection.

A considerable number of studies have been devoted to evaluating the effects of fever in the host. Before the advent of antibiotics, induced fever (hyperthermia) was used to treat such disease as syphilis, gonococcal infections, and pneumonococcal meningitis. It was suggested that hyperthermia sterilized the blood and promoted recovery. Later studies, after the discovery of antibiotics, indicated that the combination of antimicrobial therapy and fever promoted a faster cure than either modality alone. Studies with animals have also demonstrated that hyperthermia increases the victim's resistance to various bacteria and that recovery from diseases caused by these microorganisms coincides with elevated temperatures. The effects of hyperthermia are related to the actual temperature as

well as the length of time of hyperthermia. Fever may not always be beneficial. Fever may result in increased heart rate, which would be deleterious to those with compromised cardiovascular function. Fever also increases the basic metabolic rate by 7 to 8 percent for each degree Fahrenheit of temperature elevated. This usually results in negative nitrogen balance and mild to severe dehydration.

Skin or epithelial lesions are a frequent response to infection by many microorganism and result from (1) localization of the microorganisms near the body surface, (2) the effects of a microbial toxin near the body surface, or (3) an inflammatory response (hypersensitivity) to a microbial antigen. Multiple lesions that appear on the body surface are called *rashes*. The rashes produced by viruses often go through a succession of forms such as the following:

Macule. A macular lesion is flat and usually red.
Papule. A papular lesion is firm and elevated.
Vesicle. A vesicular lesion contains fluid.
Pustule. A pustular lesion contains pus, often a result of secondary bacterial infection.

Rashes that are localized on the body surface can be of diagnostic importance.

Other symptoms of disease may include cough, which is associated with respiratory disease. Intestinal diseases are usually characterized by the symptoms of vomiting, diarrhea, or dysentery (bloody diarrhea).

CONVALESCENT PERIOD. *Convalescence* refers to the recovery period from a disease. Convalescence is associated with a sharp decline in symptoms such as fever or headache associated with the disease in question. The subsiding of symptoms is correlated with maximum antibody levels in the host and usually means the pathogen is being eradicated.

Chronic Disease and Persistence

Chronic disease represents a form of microbial persistence in the host in which the symptoms of the disease are expressed over a long period of time. Many times in chronic disease the infectious agent is an intracellular parasite that localizes in phagocytes. The organism can, therefore, be transported to various organs and body sites where microbial multiplication or even lysis of microorganisms releases products that give rise to the original symptoms of disease or new symptoms. The symptoms arising during relapses are often due to hypersensitivity reactions by the host. Chronic diseases such as brucellosis and tuberculosis are believed to be due to the inability of the host to respond with any immunological efficiency. Apparently the microbial antigens are not sufficiently immunogenic to make the host produce antibodies that can eliminate the pathogen.

Some microorganisms that persist in the host give rise to mild symptoms or no symptoms and are actively shed from the host. The individual shedding these microorganisms is called a *carrier*. These microorganisms may persist in the host for days, months, or even years. The host-parasite relationship during persistence is such that microorganisms are tolerated by the host and actively multiply to high concentrations. Although shedding is characteristic of some persistent infections it does not occur in all diseases. Persistence without shedding is found during several viral infections. Microorganisms harbored by the host may be shed from the upper respiratory tract, for example, as in diphtheria, or from the intestinal tract, for example, as in cholera or typhoid fever. The carrier state will be discussed later in the chapter. Some viruses persist in the host for years during which shedding occurs intermittently. The persisting dormant state is referred to as *latency*, which will be discussed in relation to the viruses in Chapter 33.

RESERVOIRS AND SOURCES OF DISEASE

Pathogenic microorganisms, if they are going to retain their potential to cause disease, must have a place where they can maintain themselves and rep-

licate. This place of survival and replication is called the *reservoir*, and it may be animate or inanimate. Viruses, which are obligate intracellular parasites, replicate only in living cells and are harbored in a variety of living hosts. Animate reservoirs are also required for bacterial species such as the streptococci and staphylococci, even though they are not obligate parasites. Reservoirs may also be inanimate. Many microorganisms replicate in soil, water, and food, provided nutrients are available. Many bacterial species that cause disease in the hospital, such as *Pseudomonas* and *Klebsiella*, species may be found in both inanimate and animate reservoirs.

Sources of disease are those objects or places, either animate or inanimate, from which the infective agent passes to the host. Sometimes the reservoir and source are one and the same. The organism that causes pneumonia (*Streptococcus pneumoniae*), for example, is harbored by humans in the oral cavity. If the infected individual comes into direct contact (by kissing, for example) with another individual and causes disease, the reservoir and source are the same. There are also many examples in which the reservoir and source are not the same. If the reservoir of disease is the kidney of an animal, the infectious microorganism may infect a human in two ways. Humans may eat the kidneys of the diseased animal, in which case the reservoir and source are the same. The live animal may also contaminate a water supply through urination. Ingestion of the contaminated water by humans could also lead to disease, and in this instance the source of the disease would be water. The length of time that the microorganism remains viable in the source depends on environmental as well as microbial factors.

ANIMATE RESERVOIRS

Human

Humans not only represent the most important reservoir of microbial agents but in many instances they are the only reservoir. Many viral agents, such as the measles, rubella, mumps, influenza, and poliomyelitis virus, and bacterial agents, such as those causing sexually transmitted disease, whooping cough, and diphtheria are maintained only in human reservoirs.

Humans may be a source of infectious agents even though they do not exhibit any symptoms of disease. These individuals are called carriers and are of two types: convalescent and healthy. *Convalescent carriers* are those who are recovering from the disease and in whom the infectious agent remains and multiplies without causing overt symptoms. *Healthy carriers* are those who do not have the disease but still carry infectious microorganisms. Many of the microbial agents harbored by carriers are capable of replicating in inanimate sources such as food and water. Those who carry strains of *Staphylococcus aureus* that are causes of food poisoning are examples of healthy carriers. The carrier harbors the staphylococci in the nares (nostrils) and when he or she handles food contaminates it with staphylococci. The staphylococci multiply in the food and produce a toxin that when ingested causes food poisoning. The carrier state may last a few weeks, several months, or even years depending on the factors that contribute to the host-parasite relationship. Detection of the carrier state is very important in severing the chain of transmission that may involve hundreds or even thousands of individuals.

Animal

Animals, particularly domestic animals, are important reservoirs and sources of disease to humans. A disease that occurs primarily in animals and is secondarily transmitted to humans is called a *zoonosis*. *Salmonella* species are normally found in the intestinal tract of animals such as poultry or cattle. When they contaminate food ingested by humans the salmonellae can cause disease (salmonellosis). Table 13-1 describes the zoonoses. Many times when animals are reservoirs the human represents a dead end in terms of disease transmission because the disease cannot be transferred from human to human. For example, Q fever and brucellosis are diseases of animals that can be acquired through contact with the animals or animal prod-

Table 13-1. Diseases of Animals (Zoonoses) That Are Transmissible to Humans

Disease	Transmission to humans	Animal reservoir
BACTERIAL		
Salmonellosis	Ingestion of contaminated food or water	Dogs, cats, farm animals, poultry, reptiles, rodents
Brucellosis	Drinking raw milk or direct contact with animal	Dogs, farm animals, rodents
Tularemia	Ingestion of or contact with contaminated meat and arthropod vectors	Rodents and rabbits
Anthrax	Contact with contaminated animal hides or products	Farm animals
Leptospirosis	Contact with contaminated water	Dogs, cats, wild rodents, farm animals
Bubonic plague	Rat flea but human-to-human transmission in pneumonic plague	Rodents such as ground squirrels, rats, chipmunks
Rickettsial disease (scrub typhus and murine typhus)	Ticks, fleas, mites	Primarily wild rodents
Q fever	Ingestion of raw milk, inhalation of contaminated dust, or contact with contaminated animal	Farm animals (cattle, sheep, goats)
Cat-scratch fever	Scratch or bite of cat	Cats
VIRAL		
Rabies	Bite of animal or contact with infectious saliva or tissue, inhalation of aerosols	Dogs, cats, farm animals, and wild animals, particularly skunks and raccoons
Equine encephalitis	Horses to mosquitoes to humans	Horses, pheasants, domestic pigeons
PARASITIC (HELMINTHS)		
Echinococcosis	Ingestion of eggs deposited on raw fruit	Fox, dogs, cats are principal hosts, but life cycles in nature completed in voles and mice
Trichinosis	Ingestion of contaminated meat, usually pork	Pigs and bears are most important reservoirs

ucts. There are exceptions, of course, such as in the case of the disease salmonellosis. Salmonellosis may be acquired from animals but the infected human can also serve as a source of disease to other humans.

Insect

Two classes of arthropods, Insecta and Arachnida, are important in the transfer of infectious agents from one host to another. Arthropods are therefore called *vectors* in the disease process. The class Insecta includes flies, mosquitoes, fleas, lice, and true bugs; Arachnida includes ticks and mites. Insect vectors may be divided into two types: mechanical and biological. *Mechanical vectors* are invertebrate animals that carry the infectious agent on their appendages and are not involved in the life cycle of the infectious agent. Flies, for example, may pick up *Salmonella* organisms from animal feces and transmit them to humans via contaminated food. Thus the fly is not a true reservoir of the infectious agent. *Biological vectors* are invertebrate animals that serve as host and reservoir of the microbial agent. Many arthropods feed on animals and humans and during the blood feast acquire the infectious microorganism. The infected arthropod can feed on other hosts, which may include humans, and thus transmit the infectious microorgan-

Table 13-2. Major Vector-borne Diseases of Humans

Vector	Disease	Microbe classification
Mosquito	Animal parasite diseases	
Culex, Anopheles, and *Aedes* species	Filariasis	*Wuchereria* and *Brugia* species
Anopheles species	Malaria	*Plasmodium* species
	Viral diseases	
Aedes species	Dengue fever	Flavivirus
	Yellow fever	Flavivirus
	St. Louis encephalitis	Flavivirus
Culex species	Eastern equine encephalitis	Alphavirus
	Western equine encephalitis	Alphavirus
	St. Louis encephalitis	Flavivirus
Tick	Bacterial diseases	
	Relapsing fever	*Borrelia recurrentis*
	Rocky Mountain spotted fever	*Rickettsia rickettsii*
	Q fever	*Coxiella burnetii*
	Tularemia	*Francisella tularensis*
	Viral diseases	
	Colorado tick fever	Orbivirus
	Crimean hemorrhagic fever	? (ungrouped)
Mite	Bacterial diseases	
	Q fever	*Coxiella burnetii*
	Rickettsialpox	*Rickettsia akari*
Flies	Animal parasite diseases	
Deerfly	Eye worm	*Loa loa*
Tsetse flies	Sleeping sickness (African)	*Trypanosoma* species
Blackflies	Leishmaniasis	*Leishmania* species
	Onchocerciasis	*Onchocerca volvulus*
	Bacterial diseases	
Deerfly	Tularemia	*Francisella tularensis*
Muscoid flies	Yaws	*Treponema pertenue*
	Viral diseases	
Sandfly	Sandfly fever	?
	Vesicular stomatitis	Vesiculovirus
Lice	Bacterial diseases	
	Epidemic typhus	*Rickettsia prowazekii*
	Trench fever	*Rochalimaea quintana*
Fleas	Bacterial diseases	
	Endemic typhus	*Rickettsia typhi*
	Bubonic plague	*Yersinia pestis*
True bugs (reduviids)	Animal parasite diseases	
	Chagas' disease (American trypanosomiasis)	*Trypanosoma cruzi*

Source: From R. F. Boyd, *General Microbiology*. St. Louis: Mosby, 1984.

isms from one host to another. Occasionally the arthropod is required in the developmental cycle of the microbial agent. The protozoal agent that is the cause of malaria requires the mosquito to undergo its sexual cycle. In the epithelial cells of the mosquito stomach the protozoan matures and then migrates to the salivary glands. When the mosquito bites a human the pathogen is injected into the human host. The insect vectors and the diseases with which they are associated are outlined in Table 13-2.

INANIMATE RESERVOIRS

Inanimate reservoirs or sources of disease are soil, water, and food. Many microorganisms are saprophytes in soil and water, and, fortunately for us, most are not pathogenic for humans. Most fungal species, for example, exist in the soil as spores. When some of these spores are inhaled by a susceptible host, they germinate and engage in vegetative growth and produce disease. Bacteria that produce the deadliest toxins known to humans are indigenous to the soil. Species of *Clostridium* such as *C. botulinum* and *C. tetani* exist in the soil as spores. *C. botulinum* may contaminate food and under anaerobic conditions produces the toxin associated with food poisoning (botulism). If *C. tetani* spores contaminate a wound they germinate and produce a toxin responsible for the symptoms of the highly fatal disease called *tetanus*. Animal parasites such as the parasitic worms (helminths) are usually passed as eggs from the intestinal tract of the animals they parasitize. These eggs may remain in an infective state until they are picked up by another host. Very few microorganisms pathogenic for humans are capable of vegetative growth or a free-living existence in the soil.

Pure water is unable to support the growth of microorganisms, but most water contains minerals and organic nutrients that have been derived from the surrounding soil or air. Humans also contaminate waters either directly or indirectly via raw or processed sewage. Pathogenic as well as nonpathogenic microorganisms may find their way from humans or animals into water supplies. Some potential pathogens for humans live in the fish and shellfish that inhabit inland and coastal waters. Thus water supports their existence and also contributes to their potential for infecting humans.

Food is an important reservoir for microorganisms infectious for humans. Human disease may result from ingestion of the meat of the infected animal or ingestion of originally uninfected meat that has been contaminated. Several bacterial and animal parasite diseases can be acquired by humans from the ingestion of meat from diseased animals. Eggs may be infected with *Salmonella* and when eaten raw can cause human disease. Cows carry several microorganisms infectious for humans. Ingestion of infected meat or milk may cause disease in humans. Tuberculosis, brucellosis, and Q fever are the most frequently encountered diseases resulting from the ingestion of infected meat or milk; however, diseases caused by species of *Campylobacter* and *Streptococcus* can also occur. These diseases are associated with drinking raw milk. Pasteurization ordinarily destroys these organisms. Various tapeworms infect animals and are encysted in muscle. Ingestion of uncooked pork, for example, can lead to the disease called *trichinosis*.

Foods may be accidentally contaminated with potentially infectious microorganisms. Shellfish such as oysters, for example, may be harvested from waters that are polluted with human sewage. Ingestion of such foods uncooked can lead to such diseases as hepatitis. Carriers may contaminate food and be a source of disease to humans. Diseases such as salmonellosis, shigellosis, amebiasis, and cholera can be acquired in this way. Some microorganisms that contaminate foods produce toxins that when ingested cause gastrointestinal symptoms (see Transmission of Infectious Microorganisms). When properly refrigerated, smoked, canned, or pasteurized, however, foods are safe to eat and will not cause disease.

TRANSMISSION OF INFECTIOUS MICROORGANISMS

Disease transmission refers to the method of transfer of infectious microorganisms from the source (which may or may not be the same as the reservoir) to the host. Transmission may occur by contact, through the air, or by means of a vector. (Transmission by vectors was discussed under Reservoirs and Sources of Disease.) Sometimes an organism may have more than one route of transmission.

CONTACT

Spread of microorganisms by contact occurs when the potential victim has contact with the source of the microorganisms. This contact may either be direct or indirect.

Direct

Direct contact refers to transmission of microorganisms from person to person by close personal association. Handshaking, kissing, sneezing, coughing, and sexual contact represent the most usual ways that microorganisms are transferred by direct means.

Sexually transmitted diseases have become rampant in many parts of the world, especially in developed countries like the United States. To most people sexually transmitted diseases are represented by the traditional diseases syphilis and gonorrhea. Greater sexual freedom, as well as changes in sexual practices, has resulted in recent years in an increasing number of sexually transmitted diseases of unusual types. For example, chlamydial infection is now more common than gonorrhea, and the incidence of genital herpes is believed to be as high as 500,000 cases per year. Increased homosexual activity has also resulted in an increase in diseases usually considered to be primarily enteric. Enteric diseases such as giardiasis, salmonellosis, and shigellosis are now frequently encountered in the gay community. A list of the sexually transmitted diseases is presented in Table 13-3. The complications of sexually transmitted disease, especially in women, have created great concern. Increased health expenditures, loss of productivity, and human suffering have resulted from sexually transmitted disease complications such as pelvic inflammatory disease. These complications have led to loss of fertility as well as detrimental effects in the newborn.

Sneezing and coughing may also be considered a method of direct spread, provided the individuals are within a few feet of each other. The microorganisms are expelled in droplets that are carried only a few feet and then drop to horizontal surfaces. Direct spread of this kind is characteristic of measles, a viral disease.

Direct contact may also be responsible for the transmission of those diseases that are caused by the patient's own microbial flora. These infections, sometimes referred to as *endogenous*, are frequently encountered in the hospital environment. Patients, either because of examination or treatment procedures, can have indigenous microbial species transferred from one body site to another. During surgery, for example, intestinal microorganisms may be accidentally displaced from the intestinal tract into the bloodstream where they replicate rapidly and cause disease (see Chapter 38).

Indirect

Indirect transmission occurs when the infectious microorganism is carried from one person to another on various intermediate objects such as food, dust, water, or *fomites* (inanimate objects other than food or water). The intermediate objects may be contaminated from an animate or inanimate source.

WATER. Water is a common vehicle for the transmission of infectious microorganisms. An outbreak of waterborne disease is defined as an incident in which two or more persons experience similar illness after consumption or use of water intended for drinking and epidemiological evidence implicates water as the source of the illness. Community as well as noncommunity water systems may be in-

Table 13-3. Characteristics of the Sexually Transmitted Diseases

Disease	Causal agent	Type of parasite	Cause of infertility, abortion, or infection to fetus	No. of cases reported to CDC, 1984	Estimated no. of infections per year
Gonorrhea	*Neisseria gonorrhoeae*	Bacterium	Yes	822,089	2.5 million
Syphilis	*Treponema pallidum*	Bacterium	Yes	26,974	340,000
Nongonococcal urethritis	*Chlamydia trachomatis, Ureaplasma urealyticum*	Bacteria	No	NR	1.0 million
Trichomoniasis	*Trichomonas vaginalis*	Protozoan	No	NR	1.0 million
Venereal warts	Human papilloma virus	Virus	No	NR	600,000–900,000
Genital herpes	Herpes simplex virus	Virus	Yes	NR	500,000
Lymphogranuloma venereum	*Chlamydia trachomatis*	Bacterium	No	250	?
Soft chancre (chancroid)	*Hemophilus ducreyi*	Bacterium	No	646	?
Granuloma inguinale	*Calymmatobacterium granulomatis*	Bacterium	No	60	?
Candidiasis	*Candida albicans*	Fungus	Yes	NR	?
Scabies	*Sarcoptes scabiei*	Arachnid (mite)	No	NR	?
Pediculosis pubis	*Phthirus pubis*	Insect (louse)	No	NR	?
Enteric disease	*Salmonella* species	Bacterium	No	NR	?
	Shigella species	Bacterium	No	NR	?
	Campylobacter fetus subspecies *jejuni*	Bacterium	Yes	NR	?
	Entamoeba histolytica	Protozoan	No	NR	?
	Giardia lamblia	Protozoan	No	NR	?
AIDS (acquired immune deficiency syndrome)	Human T-lymphocyte virus type III	Virus	No	3,026	?

CDC = Centers for Disease Control; NR = not reportable.

volved. Many outbreaks occur in recreational areas where wells or other water sources become fecally contaminated. From 40 to 50 outbreaks, involving about 4000 to 5000 cases, occur each year in the United States. The most frequently recovered pathogen is *Giardia lamblia*, a protozoan that causes gastrointestinal disease. The infectious agents associated with waterborne disease are outlined in Table 13-4.

FOOD. A foodborne disease outbreak is defined as an incident in which two or more persons experience a similar illness, usually gastrointestinal, after ingestion of a common food and epidemiological analysis implicates the food as the source of the illness. Over 60 percent of foodborne disease outbreaks are associated with restaurants; the remainder are associated with foods eaten at home (Table 13-5). Factors that contribute to foodborne illness

Table 13-4. Infectious Agents Associated with Waterborne Disease*

Causal agent	Incubation period	Clinical syndrome
BACTERIAL		
Escherichia coli	6–36 hr	Gastrointestinal syndrome, usually diarrhea
Salmonella species	6–48 hr	Gastrointestinal syndrome, usually diarrhea
Shigella species	12–48 hr	Gastrointestinal syndrome, usually diarrhea
Campylobacter fetus subspecies *jejuni*	2–5 days	Gastrointestinal syndrome, usually diarrhea
Yersinia enterocolitica	3–7 days	Gastrointestinal syndrome, usually diarrhea
PARASITIC		
Giardia lamblia	1–4 weeks	Gastrointestinal syndrome, usually chronic diarrhea, cramps, fatigue, and weight loss
Entamoeba histolytica	2–4 weeks	Gastrointestinal syndrome, variable from acute fulminating dysentery with fever, chills, and bloody stools to mild diarrhea and abdominal cramps
VIRAL		
Hepatitis A	2–4 weeks	Fever, nausea, vomiting, dark urine
Norwalk and Norwalk-like agents	24–48 hr	Gastrointestinal syndrome, vomiting, watery diarrhea, abdominal cramps, often headache
Rotavirus	24–72 hr	Gastrointestinal syndrome, vomiting, watery diarrhea, abdominal cramps, often with dehydration
Enterovirus	5–10 days	Other syndromes than gastrointestinal: poliomyelitis, aseptic meningitis, and herpangina

*Heavy metals may also be associated with waterborne disease, but they are not included here.

are (1) improper holding temperatures for food, (2) poor personal hygiene, (3) contaminated equipment used in the processing of food, (4) inadequate cooking, and (5) food obtained from an unsafe source. The microbial agents most frequently implicated in foodborne illness are *Salmonella* species, *S. aureus*, and *Clostridium perfringens*. They are responsible for nearly 80 percent of the outbreaks in the United States. The major factor responsible for these outbreaks is improper holding temperature. Foodborne disease may be divided into two types: *food infections* and *food poisonings*. Food infections result from the ingestion of microorganisms, such as *Salmonella*, found in the food. Salmonellae produce a toxin in the victim's intestine that causes gastrointestinal symptoms. Food poisonings are the result of ingestion of toxins that were liberated during growth of microorganisms in the food. Microbial growth occurs because the contaminated food was left at an improper holding temperature. *S. aureus*, for example, may be transmitted by food handlers, and if the food is left at improper holding temperatures the microorganisms multiply and produce a toxin that, if ingested, causes gastrointestinal disease.

FOMITES. Inanimate objects (fomites) are also involved in disease transmission. Contamination of inanimate objects is a frequently encountered method of disease transmission in the hospital. Catheters, needles, and other objects may be contaminated by hospital personnel and when these devices come into contact with the patient may initiate disease (see Chapter 38).

AIR

Microorganisms cannot use air as a reservoir, but they can use it as means of dissemination. Airborne transmission implies that the organisms travel longer distances than in the direct type of transmission between individuals, which usually involves

Table 13-5. Confirmed Foodborne Disease Outbreaks,* United States, 1981

Causal agent	Outbreaks	Cases	Deaths
BACTERIAL			
Bacillus cereus	8	74	0
Campylobacter fetus subspecies *jejuni*	10	487	0
Clostridium botulinum	11	22	1
Clostridium perfringens	28	1162	2
Salmonella species	66	2456	21
Shigella species	9	351	0
Staphylococcus aureus	44	2934	1
Streptococcus group A	2	307	0
Streptococcus group D	1	24	0
Vibrio cholerae non-OI	1	4	0
Vibrio parahaemolyticus	2	13	0
Yersinia enterocolitica	2	326	0
Other	1	48	0
PARASITIC			
Giardia lamblia	1	61	0
Trichinella spiralis	7	70	1
VIRAL			
Hepatitis A	6	128	0

*Foodborne disease may also be caused by chemicals such as those produced by fish, those used as preservatives (monosodium glutamate), those derived from mushrooms, and heavy metals. The above table represents only foodborne disease associated with infectious agents.
Source: Adapted from *Foodborne Disease Surveillance*. Atlanta: Centers for Disease Control, 1983.

only a few feet. Wind currents, humidity, and other environmental factors influence the distance microorganisms can travel. Outdoor microorganisms are found in the soil and water and on vegetation. Many of these microorganisms exist as spores that travel in the wind currents or as vegetative forms that have been aerosolized and carried by currents of air. Many of the fungal diseases, for example, result from the inhalation of spores that contaminate the environment and are carried in the air.

Indoors, microorganisms are also disseminated either in dust or as droplet nuclei. *Dust* is material that has settled to surfaces and is subject to dissemination by air currents or various physical actions (shaking a dust mop, for example). The dust particles may remain suspended in the air for various lengths of time. Some microorganisms such as staphylococci and streptococci can remain viable in dust for up to three months. This is a characteristic that is more common among the gram-positive bacteria than gram-negative bacteria. The cell envelope of gram-positive bacteria is considerably thicker and more resistant to drying and ultraviolet radiation than that of gram-negative bacteria. *Droplet nuclei* are particles of mucus that have been expelled from humans by sneezing or coughing. These mucus particles contain microorganisms and because of their small size (5 μm or smaller) remain suspended in air for long periods of time. Tuberculosis is a disease that is transmitted by droplet nuclei.

Aerosols generated by spraying water into the air (e.g., at water treatment facilities or in humidifiers or water coolers) may also contain microorganisms that are infectious for humans. Legionnaires' disease, for example, is caused by a microorganism whose primary habitat is water and is transmitted to humans by aerosols.

HORIZONTAL VS. VERTICAL TRANSMISSION

Microorganisms must spread from one individual to another if they are to persist and cause disease. If an individual infected with the measles virus, for example, does not make contact with other individuals, the virus will die in the host. Microorganisms can be spread horizontally or vertically. *Horizontal spread* is the transfer of disease by everyday contact, that is, by air, water, food, contact, or vectors. *Vertical spread* involves transfer of infectious agents from parent to offspring via sperm, ovum, placenta, milk, or direct contact.

Vertical Transmission

The vertical transmission of infectious agents may occur after birth (postnatal), before birth (congeni-

tal), or during birth (perinatal). Congenital transmission involves transfer of the microbial agent across the placenta; perinatal infection occurs during passage through the birth canal. The microbial agents associated with congenital and perinatal infections are outlined in Table 13-6. Transmission across the placental membrane results in deposition of the microbial agent into the fetal blood. The infectious agent can then be disseminated to any organ or tissue of the fetus. Transmission during passage through the birth canal results initially in infection of the skin, eyes, or respiratory tract of the infant. Depending on its virulence, the microbial agent may later invade the bloodstream. The infant, after infection, often becomes a shedder of the microbial agent because of immune tolerance. The infected host may produce antibodies to the infectious agent, but they are not sufficient to neutralize the microorganism. Infections can be devastating to the newborn, resulting in malformations, damage to various organs or tissues, and even death.

Transmission after birth (postnatal) occurs when the parent (1) experiences a primary infection at or just after the birth; (2) experiences a reactivation of a latent infection (some persistent viruses, for example, are activated during pregnancy); or (3) has been shedding virus continuously during pregnancy. Hepatitis virus, for example, is carried in the blood of the mother and is transmitted during or shortly after birth. A mother who sheds microorganisms in the milk during lactation is one of the most common means of postnatal transmission. Other vehicles of transmission include feces, urine, saliva, blood, and contact with skin and mucosa.

Transmission to the offspring via the germ line is also possible. Ova and sperm may be infected with microbial agents such as viruses. These agents, provided they do no harm to ova or sperm, could conceivably be transferred to the developing embryo. This type of transmission has been observed in animals. The mouse mammary tumor virus, for example, is transmitted via sperm in some laboratory strains. Viruses, such as the herpes simplex virus, cytomegalovirus, hepatitis virus, Marburg virus, and Ebola virus, have been found in human

Table 13-6. Microbial Agents Associated with Congenital or Perinatal Disease in Infants

Agent	Consequences of infection
CONGENITAL INFECTION	
Cytomegalovirus	Hepatitis, jaundice, congenital heart disease, mental retardation
Rubella virus	Abortion, congenital malformation, deafness, cataracts, heart defects, encephalitis
Poxvirus (smallpox)	Abortion, stillbirth, congenital smallpox
Varicella-zoster virus	Low birth weight, bilateral cataracts, atrophic limbs, mental retardation, congenital varicella
Vaccinia virus	Abortion, generalized vaccinia
Poliovirus	Congenital poliomyelitis
Measles virus	Stillbirth, congenital measles
Mumps virus	Abortion, endocardial fibroelastosis
Herpes simplex virus	Abortion, excessive brain damage, hepatoadrenal necrosis
Hepatitis B virus	Neonatal hepatitis, stillbirth, abortion
Echovirus	Hydrocephalus and neurological sequelae, jaundice (most infections are asymptomatic or mild)
Coxsackievirus B	Myocarditis and central nervous system involvement
Arbovirus	Congenital encephalitis
Treponema pallidum (bacterium)	Stillbirth, abortion, saddle nose, notched teeth, central nervous system involvement
Toxoplasma gondii (protozoan)	Stillbirth, hydrocephalus, mental retardation
PERINATAL INFECTION	
Herpes simplex virus	Localized infection of oral cavity, skin, eye
Neisseria gonorrhoeae (bacterium)	Infections of the eye (ophthalmia neonatorum)
Chlamydia trachomatis (bacterium)	Inclusion conjunctivitis
Streptococcus group B (bacterium)	Pneumonia, septicemia, meningitis

semen although they have not been found to be involved in germ-line transmission. The established examples of germ-line transmission are the oncoviruses whose RNA genome is transcribed into DNA by the enzyme reverse transcriptase and is incorporated into the host's DNA (see Chapter 33).

PATTERNS OF DISEASE IN THE COMMUNITY

ENDEMIC, EPIDEMIC, AND SPORADIC DISEASE

Infectious diseases occur in a population with a particular frequency, which may be defined as *endemic, epidemic*, or *sporadic*. To determine which term applies to the disease in question one must have some idea of the history of the disease in the community. The history of the disease can be evaluated by noting when the disease has occurred and the number of persons involved. *Endemic* disease implies that the disease continues in a specified population without interruption. Cholera, for example, is a disease endemic to Southeast Asia. Diseases become endemic when an equilibrium has been established between the host and the parasite. One characteristic of endemic disease is that many individuals in the community have clinically inapparent disease. An influx of persons, for example, from a geographical area where the disease is not endemic represents a highly susceptible population. Endemic disease may also be associated with animal populations, where it is referred to as enzootic disease. An outbreak of 2 to 3 cases of diphtheria every 8 to 10 years exemplifies a sporadic disease.

When the number of new cases of disease in a defined period of time rises above its normal endemic level we speak of the disease as being *epidemic*. The epidemic usually begins with a sudden occurrence of disease in those who are susceptible and who come into contact with an infected source. Epidemics involve specific populations that may vary from a single site such as a hospital, to a city, to large geographic areas that may encompass the globe. The term *pandemic* is used to denote worldwide epidemics.

Sporadic diseases are those that occur in such an irregular pattern that their frequency cannot be calculated. For example, an individual may develop a disease after the fracture of a limb.

INCIDENCE AND PREVALENCE

The occurrence of disease in a population may be quantified by determining its incidence and prevalence. *Incidence* refers to the number of new cases in a particular population within a specifically defined time period. For example, the incidence may be expressed as the number of new cases per 10,000 or 100,000 individuals in the population per year. The incidence may also be referred to as *morbidity rate*. The number of individuals that have died as a result of disease in a specific time is referred to as the *mortality rate*. A constant check on morbidity and mortality rates by epidemiologists helps health care officials to ward off future outbreaks and to warn the public of how to avoid disease. *Prevalence* refers to the total number of cases (both new and old) of disease in a defined population within a specified time. It includes newly detected cases of disease as well as those earlier cases whose symptoms are still clinically apparent. If an individual had a disease that lasted 8 weeks, he or she would be counted once in an incidence survey. If two prevalence studies were performed in that 8-week period the infected patient would be counted both times.

HERD IMMUNITY

The rate at which an epidemic spreads and the number of individuals involved is determined by the factors that affect the communicability of the disease and the immunity of the population. Many diseases are highly communicable because of the manner in which they are spread. Respiratory diseases, for example, often spread rapidly, particularly in confined populations such as in classrooms, military barracks, hospitals, and institutions for the

infirm. Foodborne and waterborne diseases occur with great rapidity and involve large numbers of individuals because the victims have been exposed to a common source and because the incubation period is often very short. There are instances when exposure to a common source does not reveal itself as an epidemic because the incubation period for the disease is long and variable. Hepatitis B, for example, has an incubation period of several weeks. Thus the number of cases of hepatitis that have been defined are spread out over several weeks. With this type of disease it is difficult to identify the source and thus control the spread of disease.

The number of individuals who develop disease during an epidemic is also related to the immunity of the host. An individual who, either by passive or active means, is immune to the disease removes himself or herself as a potential source of infection to others (unless the person is a carrier). When many immune individuals are present in a community there exists what is called *herd immunity*. The herd immunity concept is based on the relative number of immune and susceptible individuals in a population. Immune individuals act as a barrier to the spread of infectious agents from infected persons to susceptible individuals. Even though a highly communicable disease may cause an epidemic, many nonimmune individuals will be protected because of the unlikelihood of their coming in contact with an infected person. One of the values of vaccine immunization is that enough individuals will be protected from disease to prevent its rapid spread to those in the population who have not received the vaccine.

DESCRIPTIVE EPIDEMIOLOGY

The routine surveillance of disease in a community to show its distribution is called *descriptive epidemiology*. Descriptive epidemiology takes into account (1) host factors that may contribute to susceptibility to disease, (2) the geographical areas where the host came in contact with the infectious agent, and (3) the time of the appearance of disease.

Host Factors

Many host factors, including age, sex, race, underlying disease, and immunization status, contribute to the susceptibility to disease. These variables are discussed in Chapter 12. All of them must be considered when evaluating the development of disease in a community.

Place

Knowing the place where contact between host and infectious agent occurs is very important in evaluating diseases in a community. If one is able to determine the site where contact has been made, measures can be taken to break the chain of transmission. Determining the place of contact between host and infectious agent is usually much easier in a confined environment such as a hospital than in the community, where infected individuals may be spread out.

Time

Time in relationship to the disease process can be viewed from different perspectives. One may observe seasonal trends, month-to-month variations, or long-term trends that span several years. Seasonal patterns are important for determining in which seasons diseases are more likely to occur. Respiratory diseases, for example, are at a considerably higher level in the fall and winter than in the summer months. Foodborne diseases, on the other hand, occur more frequently during the summer months because the ambient temperatures are higher, which permits microorganisms contaminating food to multiply rapidly. Long-term evaluations of epidemiologic patterns have been important in determining the periodicity of diseases such as influenza A. Influenza A reaches epidemic proportions every 2–3 years. One strain of the influenza A virus is able to bring about sufficient immunity in a community after an epidemic, but then the virus spontaneously changes its antigenic constitution (antigenic drift). The new strain is not recognized by the immune system of those who developed immunity to the first strain. Thus the entire population becomes susceptible to the new strain of virus (see Chapter 33 for more details on this type of antigenic variation).

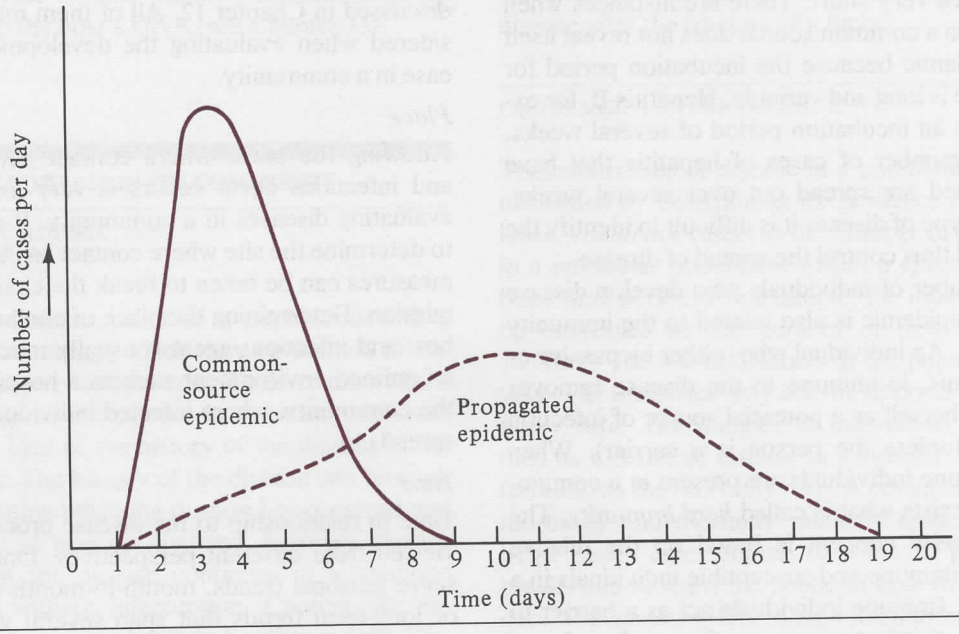


Fig. 13-1. Comparison of a common-source epidemic and a propagated epidemic. Food poisoning resulting from contamination of a food eaten by a group is an example of a common-source epidemic. Influenza, which is spread by contact between individuals, is an example of a propagated epidemic.

An epidemic can be represented graphically by plotting the number of cases of disease against time. The time scale will vary according to the incubation period of the disease. The epidemic curve begins with the *index case*, that is, the first case observed in the disease outbreak. The upslope of the curve is influenced by the incubation period, the number of exposed individuals, and the rapidity with which transmission occurs. The downslope of the curve is usually more gradual than the upslope because of the decreasing numbers of individuals susceptible to disease. Epidemics that are the result of contact with a contaminated material such as food or water are called *common-source epidemics*. In a common-source epidemic the epidemic curve rises sharply and then gradually falls off (Fig. 13-1). Epidemics that arise from person-to-person contact are called *propagated* or *contact-spread epidemics*. The epidemic curve in propagated epidemics rises slowly, has a flatter peak than the common-source epidemic, and then falls off gradually (Fig. 13-1).

LABORATORY TECHNIQUES IN EPIDEMIOLOGY

Epidemiological investigations, as we now know, provide us with valuable information that can lead to the breaking of the chain of transmission of disease and reduce its incidence. During an epidemiological investigation the symptoms of disease are often sufficient to establish the cause of disease. The causal agent of disease is also isolated in many epidemics to determine the source of disease. Many strains of an organism may exist in a community—some strains may be involved in the epidemic, but others may be nonepidemic. The problem is to separate the epidemic strains from the nonepidemic ones. *Escherichia coli*, for example,

may be involved in an epidemic of urinary tract infections. The *E. coli* strains isolated from the infected patients may be identified by several laboratory procedures. Since *E. coli* is harbored by all humans we must find a property that is unique to the epidemic strains and not to the strains found in the healthy population. A biochemical, serological, or other characteristic may differentiate the two types of strains. These differentiating characteristics are extremely important not only for determining the source of the epidemic; they also have practical value. The bacterium causing pneumonia, *Streptococcus pneumoniae*, for example, produces a capsule. Many strains of *S. pneumoniae* exist; these can be separated based on the antigenic characteristics of the capsule. Only a few strains, however, are regularly found during epidemics. To produce an effective vaccine using capsular antigens it is necessary to know which strains are the most frequently associated with disease in the community.

The principal laboratory techniques employed in epidemiological investigations are biotyping, serotyping, bacteriocin typing, antibiograms, and bacteriophage typing. Sometimes more than one technique must be used to obtain suitable results.

BIOTYPING

Biotyping refers to laboratory techniques used to identify a biological property, usually a biochemical property. The normal biochemical tests used in the clinical laboratory to identify a microorganism are sufficient for determining genus and species. Additional tests, however, may be required to separate the epidemic from nonepidemic strains. There may not be sufficient biochemical tests to differentiate strains, and tests based on nonbiochemical properties may therefore be required.

SEROTYPING

Serotyping makes use of antigenic differences between strains. These differences may be in flagellar, cell wall, or capsular antigens, depending on the organism. Serotyping is commonly used for identifying *Salmonella* and *Shigella* strains and enteropathogenic strains of *E. coli* associated with diarrhea. M protein serotyping is also useful in the epidemiology of glomerulonephritis caused by strains of *Streptococcus pyogenes*. Capsular swelling (quellung reaction) has been useful in the serotyping of *Klebsiella* species. Many laboratories do not have the antisera for typing some species, so the specimens must be sent to a reference laboratory.

ANTIBIOGRAMS

Antibiograms are tests used to determine a microorganism's susceptibility to antibiotics or other antimicrobials. Antibiograms are particularly useful in evaluating hospital epidemics because the overall susceptibility patterns of hospital-associated microorganisms are known. Antibiograms are more reliable when the acquired resistance of the organism is due to a chromosomal trait. Chromosomal traits are more stable than plasmid-associated traits since the latter can be easily transferred to other microorganisms via conjugation. The susceptibility patterns for members of the Enterobacteriaceae is generally less reliable because of the ease with which gram-negative enteric bacilli transfer R plasmids by conjugation. Antibiograms are also more useful in common-source outbreaks than in propagated epidemics because in the former each patient receives the infectious agent from the same source and there is less likelihood of the infectious agent's acquiring new resistance traits. When there is person-to-person spread there is a greater chance for conjugational transfer among microorganisms and thus the acquisition of new resistance traits.

PHAGE TYPING

Bacterial viruses are very specific in respect to the hosts they infect, and this specificity can be used as an epidemiological tool. The basis of the test is to

determine the susceptibility of the isolated clinical bacterial strains to infections by various bacteriophage sets. Phage typing systems have been internationally standardized for some bacterial species. Phage typing has one of its widest applications in the identification of strains of *Staphylococcus aureus*. Twenty-two phages are routinely used to characterize *S. aureus* strains. Phage typing has also been used for the identification of *Salmonella typhi*, the causal agent of typhoid fever. See Chapter 19 for a more detailed discussion of phage typing *S. aureus*.

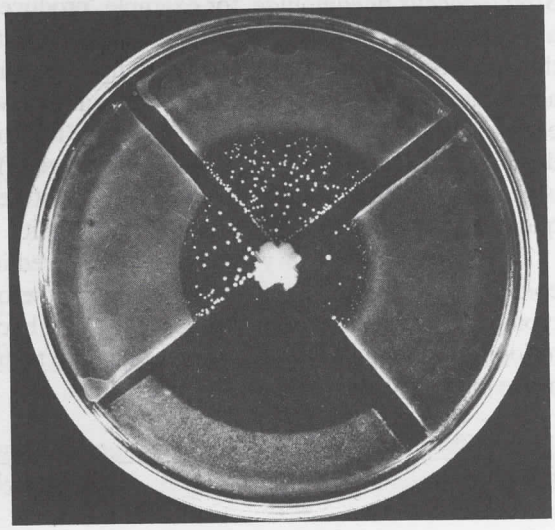
BACTERIOCIN TYPING

Bacteriocins are antimicrobial-like proteins produced primarily by strains of gram-negative enteric species (see Chapter 5). The bacteriocins are active against strains of the same or closely related species. The test is usually performed by making a single streak of a known bacteriocin-producing strain on an agar plate. The clinical isolates (about six per plate) are then streaked at right angles to the known bacteriocin-producing strain. The plate is incubated overnight, and during this period bacteriocin diffuses into the agar. The plate is then read for growth inhibition (Fig. 13-2). Bacteriocin typing has been used to identify *Serratia marcescens, E. coli, Shigella* species, and *Proteus mirabilis*. Bacteria may also be typed by their ability to inhibit indicator strains. This is a procedure used for typing *Pseudomonas aeruginosa* strains.

OTHER METHODS

Sometimes the more routinely used typing systems are not applicable to the identification of some microbial species or, when used, do not provide results suitable for differentiation. Under these circumstances the epidemiologist must use other techniques. One of the newly developed techniques for epidemiologic investigation is plasmid characterization. Techniques involving plasmids fall into two categories, direct and indirect.

Fig. 13-2. Bacteriocin typing. Colicin sensitivity of strains of *Escherichia coli*. The macrocolony of *E. coli* in the center of the plate is an active colicin producer. As it is produced, the colicin diffuses out from the colony into the medium. This colony was grown for 24 hours, after which each of the four quarters of the plate was seeded with strains of *E. coli* with various degrees of sensitivity to the colicin. After incubation, zones of inhibition proportional to the sensitivity of the strain developed. Within the zones, growth of colicin-resistant colonies can be seen. (From L. E. Hawker and A. H. Linton [Eds.], *Microorganisms: Function, Form and Environment* [2nd ed.]. London: Edward Arnold, 1979.)



Direct plasmid analysis involves hybridization of plasmid DNA from different strains, which allows the quantitative assessment of base sequence homology. Indirect techniques include agarose gel electrophoresis and restriction endonuclease analysis. In the agarose gel technique the clinical isolates are cultivated and lysed, and then the supernatant is electrophoresed on agarose gel. The gel is washed and stained by ethidium bromide, transluminated with ultraviolet light, and photographed. Plasmid size is determined by comparing gel migration distances (Fig. 13-3) of unknown plasmids with those of plasmids of known molecular size. Plasmids of the same size may have entirely different base sequences, and these differences can be de-

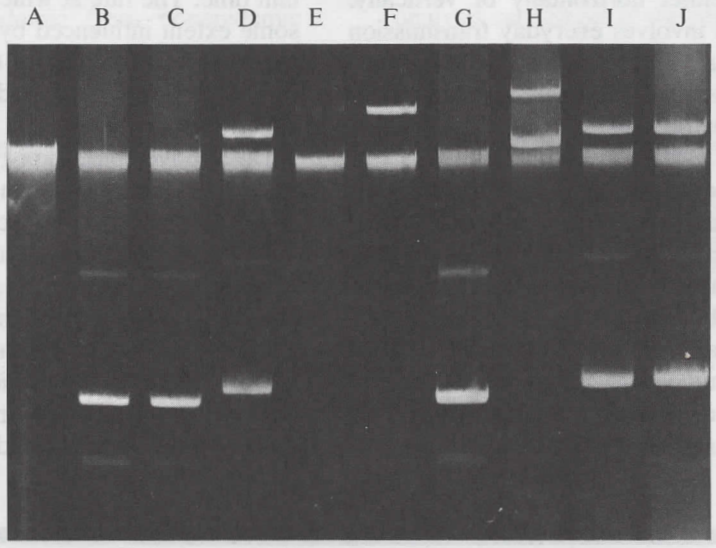


Fig. 13-3. Agarose gel electrophoresis of *Salmonella newport* from isolated cases in Pennsylvania and New Jersey, June–August 1981. Lanes B, C, and G represent the epidemic strains. (CHR = chromosomal band.) (From L. W. Riley, et al., *J. Infect. Dis.* 148:12, 1983.)

tected by using restriction endonucleases. Restriction endonucleases cleave double-stranded DNA at specific recognition sites and produce a number of plasmid fragments. If two plasmids are of the same size and yield identical patterns of fragments on restriction endonuclease analysis, they are assumed to be identical or nearly so. If the plasmids of equal size have different sequences, then different fragments will be produced from endonuclease digestion.

SUMMARY

Epidemiology is the study of the determinants, occurrence, and distribution of health and disease in a population. Disease in the host may run an acute or chronic clinical course. Acute disease runs a natural course with the immune system eventually destroying the pathogen. Acute disease can be divided into four periods: (1) incubation period, (2) prodromal period, (3) acute period, and (4) convalescent period. Chronic disease is characterized by persistence of the pathogen in the host and recurrence of symptoms. Persistence may last for weeks or even years. Shedding is relatively common in some persistent diseases such as salmonellosis and hepatitis.

The places where microorganisms are maintained and replicate are called reservoirs. Sources of disease are those objects from which the infectious agent passes to the host. Reservoirs and sources may be animate (human, animal, or insect) or inanimate (soil, water, and food).

Microorganisms may be transmitted by contact, by air, or by vectors. Transmission may be direct such as by close contact with another human, or it may be indirect such as by contact with contaminated food, water, or inanimate objects. Microorganisms can be disseminated in the air, on dust particles, or by droplet nuclei, which are mucus particles containing microorganisms. Aerosols gen-

erated in water sprays may also contribute to dissemination. Vectors such as insects also transmit microorganisms from either animals or contaminated objects. Microorganisms may be transmitted in a population either horizontally or vertically. Horizontal spread involves everyday transmission by contact—water, food, vectors. Vertical transmission refers to spread from parent to offspring, which can occur before birth, during birth, or after birth.

The patterns of disease in the community may be referred to as endemic, epidemic, or sporadic. *Endemic* disease implies that the disease is continuously present in the population. *Epidemic* refers to levels of disease above the endemic level. Epidemics that are global are referred to as pandemic. Sporadic diseases do not occur with any regularity.

Occurrence of disease in a population may be quantified by determining the incidence and prevalence. *Incidence* or *morbidity* refers to the number of new cases of disease in a specific population within a defined time period. Prevalence refers to the number of new and old cases of disease in a certain time. The rate at which a disease spreads is to some extent influenced by the number of immune individuals at the time of the epidemic. They represent a barrier to spread of disease—a concept called herd immunity.

Descriptive epidemiology uses surveillance measures that take into account (1) the host factors that affect the spread of disease, (2) the place of contact with the infectious agent, and (3) the time of the appearance of disease.

Several laboratory techniques can be used to differentiate epidemic from nonepidemic strains: biotyping, serotyping, bacteriophage typing, bacteriocin typing, and antibiograms. A recent addition to these techniques is plasmid analysis.

QUESTIONS FOR STUDY

1. What is the value of epidemiology?
2. What is the basic distinction between an acute and a chronic disease?
3. Describe the various stages of acute disease.
4. What is the difference between a healthy and convalescent carrier?
5. What are some of the major symptoms associated with the majority of diseases? Do any of them have a beneficial effect on the host?
6. What is the reason that some diseases become chronic?
7. Name some diseases that have a human reservoir. An animal reservoir.
8. What arthropods are involved in the transfer of infectious agents to humans?
9. Explain the various ways that food or water may be contaminated with infectious microorganisms.
10. What sexually transmitted diseases are potentially damaging to the fetus?
11. Which microbial agents are most frequently associated with waterborne disease? Foodborne disease?
12. How can infants become infected via vertical transmission? What microbial agents are associated with each method?
13. Make a distinction between pandemic, epidemic, and sporadic.
14. Explain the difference between the rates of incidence and prevalence.
15. What is meant by herd immunity? What role does it play in an epidemic?
16. If you were going to evaluate the distribution of disease in a community what factors would have to be taken into consideration?

17. What is the difference graphically in a common-source epidemic and a propagated epidemic?
18. What laboratory techniques are currently used to differentiate epidemic from nonepidemic strains?
19. When are antibiograms most useful in differentiating epidemic from nonepidemic strains? When are they not useful?
20. How are the indirect techniques of plasmid analysis carried out in the laboratory?

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IV. IMMUNOLOGY

14. NONSPECIFIC HOST RESISTANCE FACTORS

OBJECTIVES

To give the factors involved in nonspecific host resistance, innate immunity, and acquired immunity, and to compare their specificity, effectiveness, and duration of protection

To discuss the external factors that contribute to nonspecific host resistance

To describe the cell types and soluble factors involved in nonspecific resistance to invasive microorganisms, and to explain their roles in resistance

To describe the reticuloendothelial system and the mononuclear phagocyte system, and to point out where they differ

To discuss phagocytosis, with reference to the types of cells involved, surface versus induced phagocytosis, and the internal structural and chemical factors involved in intracellular killing

To discuss inflammation

External, First-Line Defenses
The Integument
The Normal Microflora
Internal, Second-Line Defenses
The Blood
Cellular Elements of the Blood
Neutrophils
Eosinophils
Basophils
Lymphocytes
Monocytes
Platelets (Thrombocytes)
Fluid Elements of the Blood—Plasma
The Lymphatic System
Lymphatic Vessels and Lymph
Lymph Nodes
The Reticuloendothelial System and the Mononuclear Phagocyte System
Reticuloendothelial System
Mononuclear Phagocyte System
Phagocytes
Cell Types
Dynamics of Phagocytosis
Vacuole Formation
Nonoxidative Killing and Digestion
Oxidative Killing
Secretion
Intracellular Survival of Some Microorganisms
Tissue Factors
Chemical Substances
Mast Cells
Inflammation
Glucocorticoids and Inflammation
Other Nonspecific Resistance Factors
Interferon
The Complement System
C-Reactive Protein

In Chapter 12 host resistance and immunity were cited as factors that determined whether an individual who had been exposed to an infectious agent would suffer an infection. Resistance and immunity to infectious agents and other foreign substances take three forms (Table 14-1):

1. Nonspecific host resistance. The body has defensive mechanisms that make it capable of warding off or eliminating harmful foreign agents (especially microorganisms) and substances. The principal body forces here are the integumentary system that protects the body surfaces and the tissue elements such as the reticuloendothelial system that protect against those agents that have penetrated the integument and have entered the tissues.

2. Innate immunity. In innate immunity, there is species-specific nonsusceptibility to certain microorganisms. Humans, for example, suffer natural infections of meningococcal meningitis or gonorrhea, whereas other animals do not; humans do not contract canine distemper. Species specificity is determined by anatomical and physiological properties governed by heredity—such as body temperature and the availability of appropriate cell receptors for microorganisms—and not by antigenic specificity; that is, the immune system proper is not involved. Alternative terms for innate immunity are genetic immunity, constitutional immunity, nonsusceptibility, and nonspecific immunity.

3. Acquired (adaptive) immunity. Acquired immunity results from the interactions between the immune system and antigens. Antigens are agents or substances that mostly are foreign to the body and that have certain characteristics that will be described in the next chapter. The interactions result in immune system products, namely antibodies and sensitized lymphocytes, that are specific for the antigens. The immune system products, when they subsequently react with the same antigen, destroy, eliminate, or neutralize the antigen in most cases; the reaction, however, may be harmful, as occurs in allergies. The immune system proper—its components, its interactants, the applications of its

Table 14-1. Types of Protection Against Microorganisms

Type of protection	Protection owing	Protection available	Specificity	Level of protection
Resistance	Integumentary factors; tissue elements (e.g., phagocytes, antibacterial substances)	Immediate	None or low	Variable
Innate immunity (nonsusceptibility)	Factors governed by heredity (e.g., lack of appropriate receptors for microorganisms)	Immediate	High	Absolute
Acquired immunity	Antibodies, sensitized T cells (effector T cells)	Delayed: induction period of days to weeks required in primary immune response; prompt in secondary immune response	High	Variable

products, and its beneficial and harmful actions—is the subject of the three following chapters.

EXTERNAL, FIRST-LINE DEFENSES

The integrity of the healthy body is not readily violated by foreign substances, whether they are inert materials or living forms. This inviolability is to a large extent attributable to the general nonspecific resistance mechanisms of the host.

THE INTEGUMENT

A good measure of the resistance of the host is the external, first-line defenses that prevent the penetration of a noxious agent into the tissues. Mechanical, chemical, cellular, and microbial factors protect the body surfaces.

The skin, with its keratinized surface cells, provides a tough, dense covering, a continuous surface that is interrupted occasionally by glands and hair follicles. Some of the fatty acids secreted by the glands of the skin have an antimicrobial effect. The mucous membranes are covered by a viscous, slimy secretion that traps foreign material. Antimicrobial factors such as lysozyme and white blood cells in the mucinous layer exert a protective action. The acidity and movement of food prevent colonization of the stomach with microorganisms. The filtering action of the hair of the nares and the cilia of the respiratory tract, and the flushing action of the saliva and of urine exemplify other resistance factors that prevent penetration of the tissues by foreign substances.

THE NORMAL MICROFLORA

The normal microflora of the oral cavity, the lower gastrointestinal (GI) tract, the skin, and the vagina oppose the easy establishment of new arrivals. Indigenous anaerobic bacteria of the GI tract, for example, produce volatile fatty acids that inhibit enteric pathogens of the genera *Salmonella* and *Shigella*.

Not only is the presence of normal microflora important for protection, but the balanced relationship has other effects. At issue here is the concept of superinfection. The artificial event of antimicrobial chemotherapy sometimes creates an imbalance that fosters clinical problems. A segment of the normal flora is no longer held in check by the nor-

mal competitors, which have been eliminated by chemotherapeutic agents. Broad-spectrum antibiotics may produce changes in the normal intestinal flora, which can allow an overgrowth of microorganisms that are resistant to the antibiotics, such as *Candida albicans*, and an enteritis results. Germ-free animals indirectly provide an insight into the protective role of the normal microflora. Germ-free animals are more susceptible than others to infections, including infections by microorganisms that are not habitually pathogenic. The gradual development of, and colonization with, the normal microflora appears to activate and heighten the antimicrobial resistance mechanism of the host.

INTERNAL, SECOND-LINE DEFENSES

Once foreign agents penetrate the first-line barriers, nonspecific containment is largely through the interactions of certain elements of the blood and the reactions of the vascular system, the reticuloendothelial system (RES), the lymphatic system, and tissue factors. The reactions are best evident in the inflammatory response, which can be evoked by almost any foreign substance. Phagocytic cells of the blood, the RES, and the lymphatic system have the crucial defensive function of ingesting and destroying foreign substances. The internal nonspecific resistance forces and responses of the cellular and fluid elements of the blood, the lymphatic system, the RES, phagocytes, tissue factors, and inflammation are described next. It should be understood that most of the systems, factors, cells, and responses that are to be described here under nonspecific resistance are also involved in the acquired immune responses that will be described in the next three chapters.

THE BLOOD

Besides distributing oxygen and nutrients and channeling wastes, the blood serves as a source, and a means of conveyance, of cellular and fluid elements that are important in nonspecific resistance and in immunity. The cellular and tissue elements to be described are not only operative in the blood but also readily enter the tissues under a variety of conditions, for example, in response to the presence of microorganisms in the tissues.

Cellular Elements of the Blood

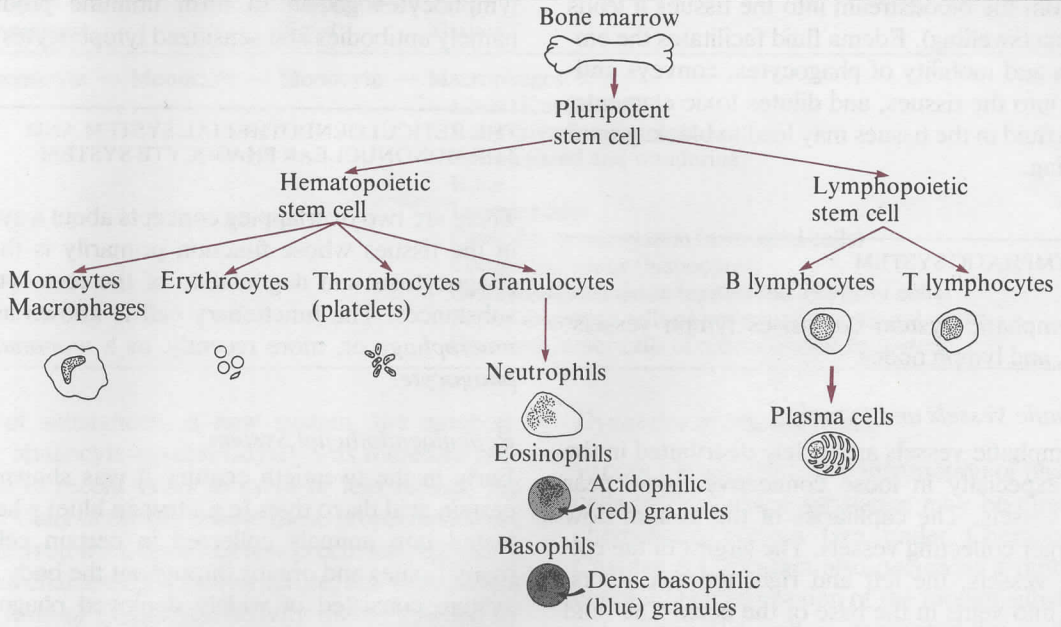
The cellular elements of the blood include erythrocytes, granulocytes, lymphocytes, monocytes, and platelets (thrombocytes) (Fig. 14-1). The blood granulocytes are subdivided into neutrophils, eosinophils, and basophils. The differentiation is based on developmental, morphological, and staining characteristics. The granules are aggregates of hydrolytic enzymes and other active degradative factors (see under Phagocytes).

NEUTROPHILS. The neutrophils, also referred to as microphages or polymorphonuclear cells (the latter abbreviated to PMNs or PMNLs), are the most numerous of the white blood cells. They and the macrophages are the principal phagocytic cells of the body. Their function as phagocytic cells will be described later in this chapter under Phagocytes. Neutrophils are the predominant cell type in acute inflammation.

EOSINOPHILS. Eosinophils are abundant in certain allergic reactions and in some infections that are caused by animal parasites. They are believed to be instrumental in controlling allergic reactions because they contain antagonists to vasoactive agents. The antagonists degrade mediators such as histamine that affect blood vessels. Eosinophils have the distinctive function of destroying the larvae of parasites from the genera *Ascaris, Schistosoma*, and *Trichinella*. They produce a small cationic protein called major basic protein (MBP) that disrupts the cuticle of parasites.

BASOPHILS. Basophils contain vasoactive substances such as histamine and slow-reacting substance (SRS), the anticoagulant heparin, and eosinophil chemotactic factors. Basophils resemble mast cells (to be described under Tissue Factors)

Fig. 14-1. Cell types involved in resistance and immunity.



because both have receptors on their surfaces for the Fc portion of the IgE class of immunoglobulin and because both contain mediators that affect blood vessels and intensify inflammation.

LYMPHOCYTES. Lymphocytes are the second most numerous cell type of the blood, they are the circulating cell type in the lymph, and they densely populate the thymus, the spleen, and lymph nodes. The role of lymphocytes in nonspecific resistance is not clearly understood, even though they are a prominent cell type in chronic inflammation. Lymphocytes have the central role in immunity and hypersensitivity, as the next three chapters will show.

MONOCYTES. Blood monocytes are regarded as immature macrophages that migrate from the circulation into the tissues where they enlarge and differentiate into macrophages.

PLATELETS (THROMBOCYTES). Platelets are small cell-like structures that do not contain a nucleus. They have several roles in hemostasis (the stopping of bleeding). They seal off breaks in blood vessels by aggregating where the continuity of the endothelium (the cell layer that lines blood vessels) has been broken. Platelets help collect the 13 blood clotting factors and facilitate their interactions. Once a clot is formed, platelets cause contraction of the clot (called *clot retraction*) to prevent obstruction of the vessel. Like basophils and mast cells, platelets contain mediators that are important in allergic reactions.

Fluid Element of the Blood—Plasma

The fluid portion of uncoagulated blood is called *plasma*. Plasma contains many substances: proteins, carbohydrates, lipids, water, hormones, salts, and gases. The principal proteins are albumin, fibrinogen, and globulins. Fibrinogen is converted into fibrin in blood coagulation and in inflammation. Globulins have their greatest relevance in acquired immunity; as immunoglobulins, they function as the antibodies of the so-called hu-

moral immunity. Various complement (see Chap. 15) components are also globulins. When plasma exits from the bloodstream into the tissues it leads to edema (swelling). Edema fluid facilitates the emigration and mobility of phagocytes, conveys antibodies into the tissues, and dilutes toxic elements. Edema fluid in the tissues may lead to blockage and blanching.

THE LYMPHATIC SYSTEM

The lymphatic system comprises lymph vessels, lymph, and lymph nodes.

Lymphatic Vessels and Lymph

The lymphatic vessels are widely distributed in the body, especially in loose connective tissue near blood vessels. The capillaries of the system flow into larger collecting vessels. The largest of the collecting vessels, the left and right thoracic ducts, empty into veins in the base of the neck. The fluid in the system, lymph, transports foreign materials such as bacteria to lymph nodes. The lymph vessels also absorb and transport ingested fats, and they recover fluids that have escaped from blood vessels into the tissues and return the fluids to the blood. Lymphocytes are the principal cell type of the system.

Lymph Nodes

Lymph nodes are ovoid, encapsulated structures that are strategically located throughout the body. Lymphocytes are the most numerous cell type. Other cell types that are present are arranged on a network of reticular fibers and reticular cells (collectively called a reticulum). Macrophages are plentiful, and they increase in numbers and activity as needs arise. There is also a system of cells whose function appears to be to capture foreign material. Functionally lymph nodes carry out major defensive and immunological functions as they filter the lymph fluid. Typically in their sojourn through lymph nodes, foreign materials—microorganisms, for example—are captured, killed, and degraded by macrophages. The foreign material within the macrophage is modified to make it antigenic when it is presented to lymphocytes. The lymphocytes go on to form immune products, namely antibodies and sensitized lymphocytes.

THE RETICULOENDOTHELIAL SYSTEM AND THE MONONUCLEAR PHAGOCYTE SYSTEM

There are two overlapping concepts about a system in the tissues whose function primarily is that of phagocytosis and degradation of the endocytosed substances. The functionary cell is known as the *macrophage* or, more recently, as a *mononuclear phagocyte*.

Reticuloendothelial System

Early in the twentieth century it was shown that certain acid diazo dyes (e.g., trypan blue) when injected into animals collected in certain cells in many tissues and organs throughout the body. This system consisted of widely deployed phagocytic cells and sheets and fibers that formed part of the structural matrix of spleen, bone marrow, lymph nodes, and other organs and tissues. Some of the phagocytic cells are fixed (sessile) in the reticulum (network of loose connective tissue fibers) and as lining cells (endothelial cells) of capillary widenings (sinusoids) (Table 14-2). The fixed cells are particularly prominent in lymph nodes, spleen, liver, and bone marrow. Some of them have more specific designations. The Kupffer cells of the liver sinusoids, the "dust" cells of the lungs, and the microglia of the central nervous system are *fixed macrophages*. Other cells of the RES are termed *wandering cells*. Via ameba-like locomotion they seek out foreign substances, frequently directed in their movement by chemotactic stimuli.

Mononuclear Phagocyte System

The unifying theme of the RES was its function in phagocytosis. It came to be realized, however, that many of the cells of the RES system, such as the lining cells of sinusoids and the fibroblastic reticular cells, were weakly phagocytic and differed from the macrophages and monocytes in their recogni-

Table 14-2. Mononuclear Phagocytes—Maturation and Compartmentalization

Bone marrow	Peripheral blood	Tissues
Promonocyte → Monocyte	→ Monocyte	→ Macrophages
		Liver (Kupffer cells)
		Lung (alveolar macrophages)
		Spleen (fixed and wandering)
		Bone
		Lymph nodes
		Central nervous system (microglial cells)
		Connective tissue (histiocytes)
		Granulomatous tissue (epithelioid and giant cells)
		Freely migrating cells of peritoneal and pleural cavities
		Germinal center cells of reticuloendothial system

tion of substances. A new system, the mononuclear phagocyte system (MPS), was therefore proposed in recent years to more or less replace the RES. Cells of the MPS have these properties: They are derived from bone marrow precursor cells, they have a characteristic cell structure, and they have a high level of phagocytic activity that is assisted by antibodies, complement components, and lymphokines (these three will be explained in the next chapter).

PHAGOCYTES

Phagocytes are cells that are specialized for ingesting, killing, degrading, and disposing of microorganisms, damaged substances and debris of host origin, foreign cells and substances, and circulating immune complexes (see Table 15-1).

Cell Types

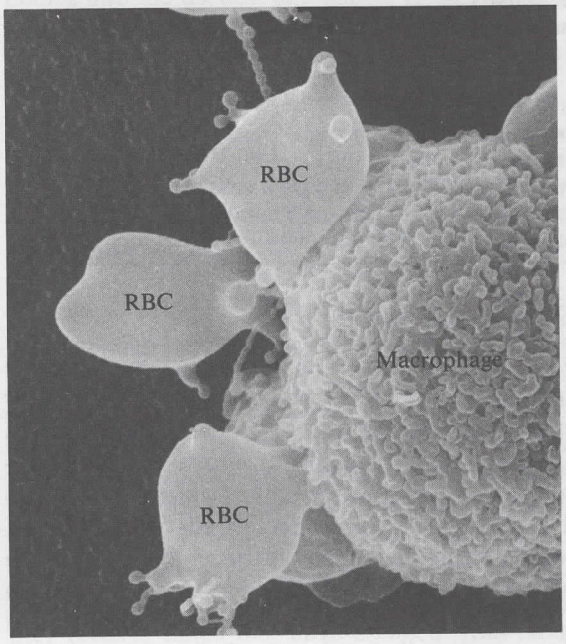
Elie Metchnikoff described phagocytosis in 1883. He proposed that certain cells displaying an ameboid motility and locomotion were the body's principal mode of defending itself against infection. Although there are other cell types that are phagocytic (e.g., eosinophils), most of the phagocytic activity is carried out by neutrophils and macrophages—in fact to such a degree that they are both coming to be referred to as the "professional phagocytes."

Dynamics of Phagocytosis

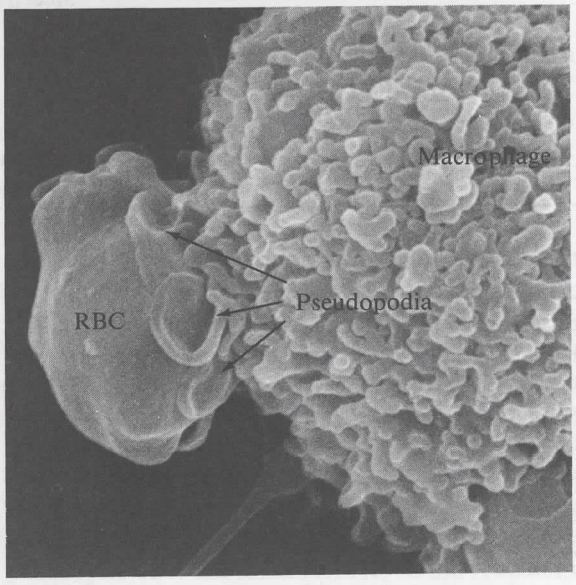
VACUOLE FORMATION. The locomotion of phagocytic cells to the foreign substance may be directed by chemotactic factors (see under Chemotaxis, in Chapter 15). Contact also occurs as a random encounter. The adsorption of the foreign substance to the phagocytic cell is facilitated by antibodies, complement fragments, and receptor sites on the phagocyte surface (see under Opsonization, in Chapter 15). In the actual process of phagocytosis the cytoplasmic membrane of the phagocyte engulfs (surrounds) the particle, and the involved segment of the membrane is pinched off (Fig. 14-2). This action creates an internalized vacuole that contains the particle. The membrane-surrounded vacuole with the contained particle is termed a *phagosome*. Killing and digestion of the ingested particle takes place by nonoxidative or oxidative mechanisms.

NONOXIDATIVE KILLING AND DIGESTION. The cell organelle called the lysosome (Fig. 14-3) is the major functionary in the process of killing and digestion. "Resting" lysosomes (primary lysosomes) are membrane-enclosed bodies that contain a collection of acid hydrolases, which are enzymes that act under slightly acid conditions. In the digestion process the membrane of the lysosome fuses with the membrane of the phagosome to form a new structure, now called a *phagolysosome* (secondary ly-

Fig. 14-2. Phagocytosis of erythrocytes by macrophages. A. Three erythrocytes (*RBC*) coated with antibody are attached to a macrophage. (×8600, before 19% reduction.) B, C. Pseudopodia of the macrophage gradually envelop an erythrocyte. (×13,000, before 16% reduction [B] and 19% reduction [C]). D. Three internalized erythrocytes have sunk to different degrees into the macrophage. (×12,000, before 19% reduction). (From J. M. Orenstein and E. Shelton, *Lab. Invest.* 36:363, 1977.)



A



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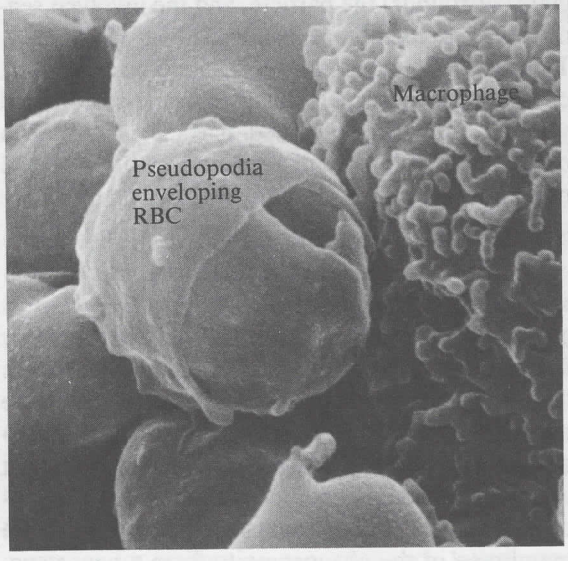

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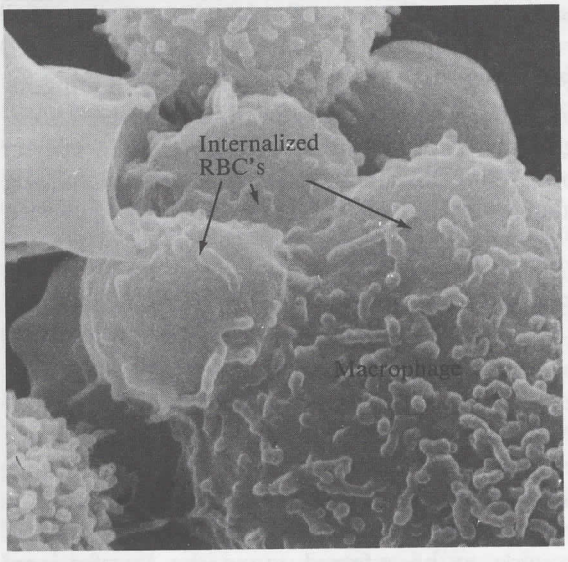

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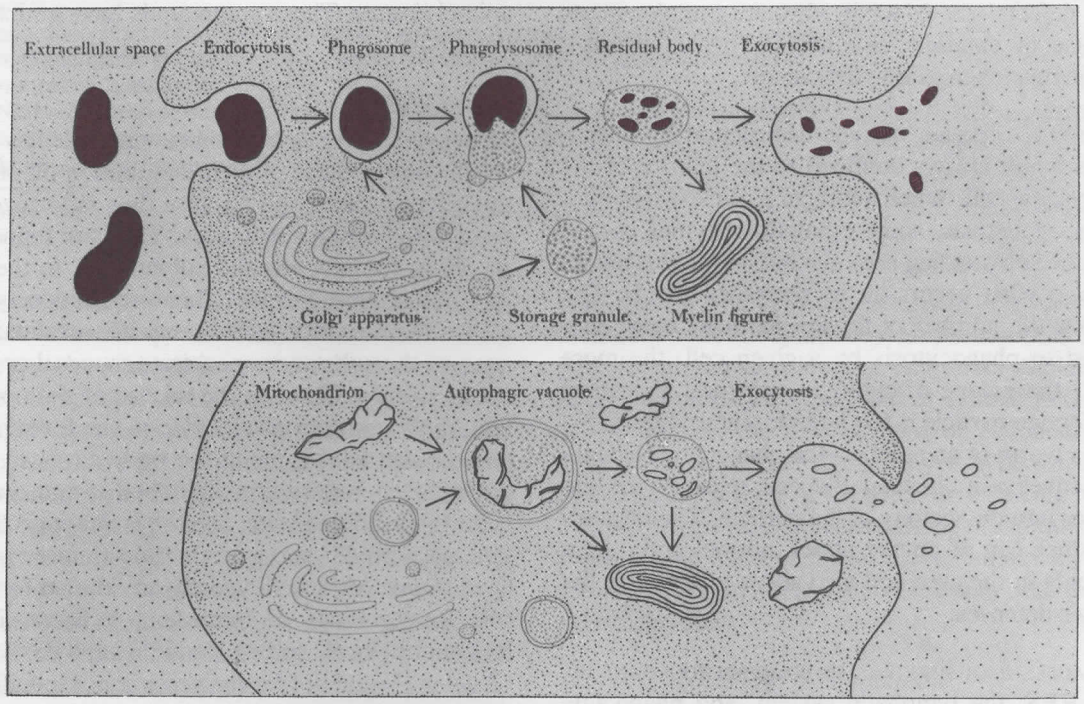

Fig. 14-3. Structures and activities involved in lysosomal function. Degradation of both extracellular (*top*) and native (*bottom*) substances is a major function of lysosomes. (From G. Weissmann, *Hosp. Pract.* 3:31, 1968. Also reprinted from chapter by G. Weissmann in R. A. Good and D. W. Fisher [Eds.], *Immunobiology*. Copyright © 1971, Sinauer Associates, Sunderland, MA.)

sosome). The lysosomal enzymes, walled off from the normal constituents of the phagocytic cell by the phagolysosomal membrane, proceed to digest the particle.

Not all cytoplasmic granules are lysosomes. Other granules contain basic proteins that are bactericidal. Macrophages usually contain only a few granules until lymphocytes activate them.

OXIDATIVE KILLING. There is a chain of chemical events involving toxic oxygen products that leads to the death of phagocytosed microorganisms: hyperpolarization (increase in electrical charge) of the phagocyte cell membrane, generation of the superoxide anion (O_2^-), conversion of most of the superoxide to hydrogen peroxide (H_2O_2), and formation of hypochlorite in the vacuole. Phagocytes become hyperpolarized about 5 seconds after contact with the foreign material. The phagocytes move cations such as sodium out of the cell, causing a negative charge on the inside of the cytoplasmic membrane. Next, surface oxidase converts molecular oxygen from the serum into the superoxide anion. The electron involved in the conversion derives from reduced nicotinamide-adenine dinucleotide or nicotinamide-adenine dinucleotide phosphate. Most of the superoxide is then converted into hydrogen peroxide. The hydrogen peroxide within the phagolysosome reacts with chloride ion (or other halide ions) to form the bactericidal substance hypochlorite (as in bleach). The formation of hypochlorite from H_2O_2 and the chloride ion takes place in the

presence of the lysosomal enzyme myeloperoxidase. Inherited defects occur in this chain of events (see under Immunodeficiencies in Phagocytes, in Chapter 17). Individuals with such defects experience serious infections even with nonpathogens. The defects are found in chronic granulomatous disease of children, in Chédiak-Higashi disease, and in myeloperoxidase deficiency.

SECRETION. Foreign particle destruction by phagocytes is not always confined to the interior of the phagocytic cell. When a foreign particle is undergoing phagocytosis, some cytolytic enzymes escape into the surrounding area in a manner that is analogous to what occurs in the secretory glands. The greater the number of foreign particles being subjected to phagocytosis by a given cell, the more likely the escape of cytolytic enzymes. Indeed, if the foreign particle is too large for internalization—say, a worm or an ameba—the phagocytic cell cannot envelop it; instead the cell releases the enzymes into the vicinity of the particle. The released enzymes can of course attack normal body components in the area and also serve to mobilize the body's defenses.

INTRACELLULAR SURVIVAL OF SOME MICROORGANISMS. The RES, the lymphatic system, and phagocytic cells normally keep the blood and tissues free of microorganisms. Some microorganisms, however, survive within phagocytes or lymphatic tissue. They may multiply there and be transported to other parts of the body. Persistent smoldering infections of lymphatic tissue, classically the tonsils and adenoids, may necessitate surgical removal of the affected tissue.

TISSUE FACTORS

Chemical Substances

There are certain less well defined antimicrobial chemical substances that contribute to nonspecific host resistance. The long-chain fatty acids of the skin and lysozymes found in saliva, tears, and nasal secretions have already been mentioned. Others reside in phagocytic cells and bear such names as *phagocytin* and *leukin*. A polyamine named *spermine*, found in semen, is bactericidal. Gram-positive bacteria are killed by *betalysin*, which is produced during clot formation. Basic polypeptides high in lysine and arginine content are present in animal tissues. These compounds have antibacterial activity.

Mast Cells

Mast cells are tissue cells that are regarded as "sentinel" cells because of their distribution on or near the body surfaces and because they recognize and react with foreign substances. They are found primarily in the mucosa and submucosa, in the dermis, and around venules. Their recognition system allows them to recognize "nonself" substances as soon as the substances penetrate mucosal or cutaneous surface. Through the release of chemotactic factors they attract phagocytes to the foreign substances that have entered the body surfaces, they facilitate the importation of antibodies and complement to the affected site, and they release proteases that purportedly serve to debride (remove) damaged tissue. Mast cells mostly are known for their role in the types of immediate hypersensitivity known as local and systemic anaphylaxis (see Chapter 17) and not for their role in nonspecific immunity.

INFLAMMATION

Infection, injury, and irritants evoke a series of interdependent reactions that serve to contain, destroy, and remove the intruder. The reactants are largely the components of the systems and factors described in the preceding paragraphs. Their combined reaction is manifest most often in the inflammatory response. Internal nonspecific resistance has many facets. If the irritant is a small group of bacteria, a single phagocyte may well dispose of them in an undetected manner. The more complex the problem, the greater the involvement of the resistance components. Quite commonly the concerted forces give rise to an overt response called *inflammation* (Table 14-3).

Table 14-3. Types of Substances That Incite an Inflammatory Response*

ENDOGENOUS
1. Nonimmmunogenic cell and tissue debris of self origin—"worn-out," devitalized, traumatized cells and tissues
2. Immunogenic substances of self origin—involved in autoimmune diseases

EXOGENOUS
1. Nonimmunogenic inert physical materials—talcum, asbestos fibers, splinters, pieces of metal
2. Nonimmunogenic chemical agents—drugs, various organic and inorganic chemicals
3. Immunogenic substances
   a. Nonmultiplicative (innocent) immunogens—foreign proteins (as in foreign serum), transfused and transplanted cells and tissues, pollens, drugs
   b. Multiplicative immunogens—microorganisms

*Substances that are immunogenic elicit responses by the immune system.

Among the responses to the initiating agent is increased vascular permeability. Vasoactive substances such as histamine, prostaglandins, kinins, and serotonin open and close vessels in response to the triggering mechanisms. Only about 10 percent of capillaries are active under normal conditions. Dilatation and increased permeability of the blood vessels allow for a greater concentration of the defensive elements. The egress of neutrophils and blood monocytes from the vessels is facilitated. The activated clotting elements of the plasma deposit fibrin to localize the irritant and to provide a support for pursuing phagocytes. Phagocytic cells of the circulating blood and wandering macrophages of the tissues are drawn to the area by chemotactic factors. Aided by antibodies and complement factors, they attach to and ingest and digest the offending agent.

The plasma and the cells from the blood that pour out into the tissues are referred to as an *exudate*. When the inflammatory process is of short duration, the inflammation is called *acute*. The cell type characteristic of acute inflammation is the neutrophil. The number of neutrophils increases promptly in response to certain infectious agents in a process called *leukocytosis*. Extensive accumulations of white blood cells—living, dying, and dead—especially neutrophils—are the major components of pus. Circumscribed accumulations of pus are termed *abscesses*.

Chronic inflammation occurs in response to irritants that are difficult to digest or that are not highly provocative. The cells characteristic of chronic inflammation are the lymphocytes, plasma cells, and reticuloendothelial cells. The end effect of chronic inflammation may be chemical or cellular excision of the irritant, the development of cell-mediated immune response (including delayed hypersensitivity), a patching over of the area, or a fencing in of the affected zone. Connective tissue proliferation is a prominent feature in chronic inflammatory responses. This serves to wall off the area or to patch a damaged area.

Glucocorticoids and Inflammation

Since glucocorticoids have wide-ranging effects on leukocytes and are widely used as anti-inflammatory agents, a few remarks about their actions are appropriate here.

Glucocorticoids affect virtually every cell type involved in inflammation. They cause a decrease in the peripheral blood monocytes, lymphocytes, eosinophils, and basophils and an increase in peripheral blood neutrophils. The major effects of glucocorticoids on leukocyte functions occur at the inflammatory focus. Glucocorticoids have a profound influence on macrophages. They deplete blood monocytes (the forerunners of tissue macrophages) more than any of the other blood cell types. They inhibit macrophage activation and the consequent secretion of collagenase, elastase, and plasminogen activator, which are associated with tissue destruction and fibrin formation at the inflammatory site. Glucocorticoids, while prompting

an increase of neutrophils, reduce their response to chemotactic stimuli so that the neutrophil traffic is random rather than zeroing in at the site of inflammation. The steroids also interfere somewhat with phagocytosis by the neutrophils and more so with the intracellular killing of ingested microorganisms.

OTHER NONSPECIFIC RESISTANCE FACTORS

Other nonspecific factors are not conveniently portrayed within the context of the inflammatory response and the systems contributing to it.

Interferon

A variety of agents induce host cells to produce interferon, a protein that has its greatest significance as an inhibitor of virus replication (see Chapter 33).

The Complement System

The complement system is a complex of serum proteins that act in a sequence once they are activated by antigen-antibody complexes or by high molecular weight carbohydrates (polysaccharides). The complement system is described in greater detail in Chapter 15. Complement has a number of biological activities, among which is a key role in nonspecific resistance. When the complement system is activated, as the sequence moves along its pathway, fragments are generated that have biological activities. Some fragments (anaphylatoxins), through their effect on mast cells, cause local vasodilation, contraction of smooth muscles, edema, and changes in blood clotting—all beneficial in coping with an irritant; the blood supply to the affected area increases, and plasma enters the tissue spaces, bringing with it antibodies and more complement components. Some fragments (chemotaxins) attract phagocytes to the area of irritation. Some fragments (opsonins) coat the surface of the irritant, promoting phagocytosis. Components of the later portion of the complement sequence can bring about structural and functional changes of cell membranes, an action that causes cell lysis and cell death.

C-reactive Protein

C-reactive protein is a beta globulin not found in the serum of normal individuals. It appears in the serum when there is inflammation anywhere in the body. It is believed to have bactericidal effects. The C-reactive protein test is a laboratory precipitation test used as a sensitive indicator of inflammation (see Chapter 16).

SUMMARY

First line nonspecific host resistance is provided by the intact integument (skin and mucous membranes) and by substances, cells, and structures on its surface such as mucus, keratin, enzymes, fatty acids, normal microflora, and cilia. Once foreign agents or materials penetrate the first-line barriers, nonspecific containment is largely through the interactions of elements of the blood; the reactions of the vascular, reticuloendothelial, lymphatic, and complement systems; and through tissue factors. Phagocytic cells have the crucial defensive functions of ingesting and destroying foreign substances. Particles ingested by phagocytes are surrounded by a piece of cytoplasmic membrane that coalesces with lysosomes to form a phagolysosome. Hydrolytic enzymes of the lysosomes digest the particles. Oxidative mechanisms that generate toxic oxygen products also destroy ingested microorganisms. Other internal defenses include antimicrobial chemical substances such as phagocytin, leukin, spermine, betalysin, and several basic polypeptides. Mast cells, most of which reside near body surfaces, attract phagocytes and facilitate the importation of antibodies and complement.

Infection, injury, and irritants evoke a series of interdependent reactions that seek to contain, destroy, and remove the intruder. The various defensive elements are focused on the intruder or injury and often produce responses that collectively are called inflammation.

QUESTIONS FOR STUDY

1. Differentiate among nonspecific host resistance, innate immunity, and acquired immunity.
2. Describe how the integument fulfills its role as the first line of resistance.
3. Give the name of the white blood cell type that matches the description:

   Contains antagonists to vasoactive agents
   Predominant cell type in acute inflammation
   Central role in acquired immunity and in hypersensitivity
   Also called thrombocyte
   Differentiates into a macrophage when it enters the tissues
   Destroys larvae of certain parasitic worms
   Most numerous white blood cell type in the blood
   Has a major role in hemostasis
   A major phagocytic cell
   Resembles mast cell in function

4. Which cell type is the principal phagocyte in the tissues?
5. The phagocytic system of the tissues for many years has been called the _____ system even if it had other functions. Recent proposals say that only those cells that are truly phagocytic should be included and that a more apt name for the tissue phagocytic system would be the _____ system.
6. Describe what takes place in the sequence of events that leads to the destruction of a phagocytosed bacterium.
7. Name some tissue factors that have antimicrobial activity.
8. Give an overview of what occurs in an inflammatory response.

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15. ACQUIRED IMMUNITY: COMPONENTS AND FUNCTIONS OF THE IMMUNE SYSTEM

OBJECTIVES

To discuss the scope of immunology, with an awareness that the field of immunology is concerned with more than resistance to infectious disease

To describe the physical, biochemical, and biological characteristics of an antigen; to differentiate among antigens, antigenic determinants, haptens, and carrier molecules, and between immunogenicity and reactivity

To compare and contrast the two limbs of the immune response, that is, humoral versus cell-mediated, with respect to the types of cells involved, the mechanisms of biological activity, and the relative importance of each with regard to different immunological phenomena

To explain the role of macrophage cells in the development of both humoral and cell-mediated immunity

To discuss the stages of B cell maturation from bone marrow stem cells; to characterize the cell at each stage of differentiation

To describe the general structure of antibodies and differentiate between the specific structures, characteristics, and biological activities of antibodies belonging to the five major classes

To describe the classic and alternative pathways of complement activation in general terms; to enumerate the biological activities of complement

To discuss the biological activities of the various T cell subpopulations, their mechanisms of action, and the role of lymphokines

To discuss the biological importance of histocompatibility antigens and the major histocompatibility complex (HLA), emphasizing the role of histocompatibility antigens in the recognition of foreign antigenic determinants by T cell populations

To differentiate between active and passive immunity and between naturally acquired and passively acquired immunity

To discuss the dynamics of an active humoral response, emphasizing the differences between primary and secondary responses and the effects of adjuvants on the dynamics observed

Introduction to the Concepts of Immunology
Development and Scope of Immunology
Factors Involved in an Immune Response
Antigens
Immunogenicity and Reactivity
Typical Antigens
Physical Makeup: Particulate and Soluble Antigens
Attributes of Antigens
Antigenic Determinants
Haptens
Thymus-Dependent and Thymus-Independent Antigens
Antigen Processing, Modification, and Presentation by Accessory Cells
Genetic Restrictions in Antigen Recognition
Macrophage Interleukin-1 as Amplifier
B Lymphocytes
Ontogeny of Two Major Lymphocyte Populations—B Lymphocytes and T Lymphocytes
Differentiation and Maturation of B Cells
Immunoglobulins (Antibodies)
Five Classes of Immunoglobulins Isotypes
Membrane-Bound Immunoglobulins as Antigen Receptors and Secreted Immunoglobulins as Effectors of Humoral Immunity
Clonal Selection Theory
Structure of the Basic Immunoglobulin Molecule
Heavy and Light Chains
Domains
Variable Region of the Fab Portion
Hinge Region
Constant Region (Fc Region)
Heavy Chain Switch
Immunoglobulin Polymers
Biological Properties of the Five Immunoglobulin Classes
Detection of Antibodies
Antibody Diversity
Heavy Chain Switch During Antigen-Driven Maturation of B Cells: S/S Recombination
Epilogue
T Lymphocytes (T Cells)
Ontogeny, Differentiation, and Maturation
T Cell Surface Antigens
The Relationship Between Histocompatibility Antigens and the Immune System
Background—Transplantation Antigens
Major Histocompatibility Complex and Immune Responsiveness
T Cell Antigen Receptors
Differences Between T Cells and B Cells
Functional Roles of T Cells—Effectors and Immunoregulators
T Effector Cells
Cytotoxic T Cells
Delayed-Type Hypersensitivity T Cells
Lymphokines
T Regulator Cells
Regulation of the Immune System
Lymphocytes With Killer Functions
The Complement System
Nature of the Complement System
Classic Complement Pathway
Alternative or Properdin Pathway
Biological Activities of Complement
Cytolysis
Opsonization
Chemotaxis
Anaphylatoxin—Mediator of Inflammation and Hypotension
Immune Adherence
Regulation of the Complement System
Complement in Diagnostic Procedures
Types of Immunity
Innate Immunity
Acquired (Adaptive) Immunity
Types of Acquired Immunity
Collation
Immunization Practices
Active Immunization
Prolongation and Intensification of the Immune Response
Dynamics of the Immune Response
Primary Immune Response
Secondary Immune Response
Passive Immunization

INTRODUCTION TO THE CONCEPTS OF IMMUNOLOGY

Immunology is concerned with the specific responses of the body to foreign substances and in lesser degree with the responses to substances from the body that it fails to recognize as "normal self." The preceding chapter described the nonspecific resistance factors. Nonspecific resistance alone, however, is inadequate, as is revealed in persons with defective immune systems (see Chapter 17). They have increased susceptibility to disease, especially infectious disease. The immune system is set in motion during the early nonspecific resistance encounters. Many of the cell types and systems associated with nonspecific resistance are involved in acquired immunity (specific resistance), especially as the mediators of the final effects on the foreign substance. Upon entrance of the foreign antigenic substance into the body, the immune system "recognizes" the foreign substance as not being "self," processes the substance, and gradually produces antibodies or lymphocytes that subsequently interact specifically with it. About one week is required after the first contact for the immune system to deal effectively with the substance in terms of being able to neutralize, destroy, or remove it. Once the immune system is primed by the first encounter, it reacts with greater velocity in subsequent engagements with the same substance (antigen).

DEVELOPMENT AND SCOPE OF IMMUNOLOGY

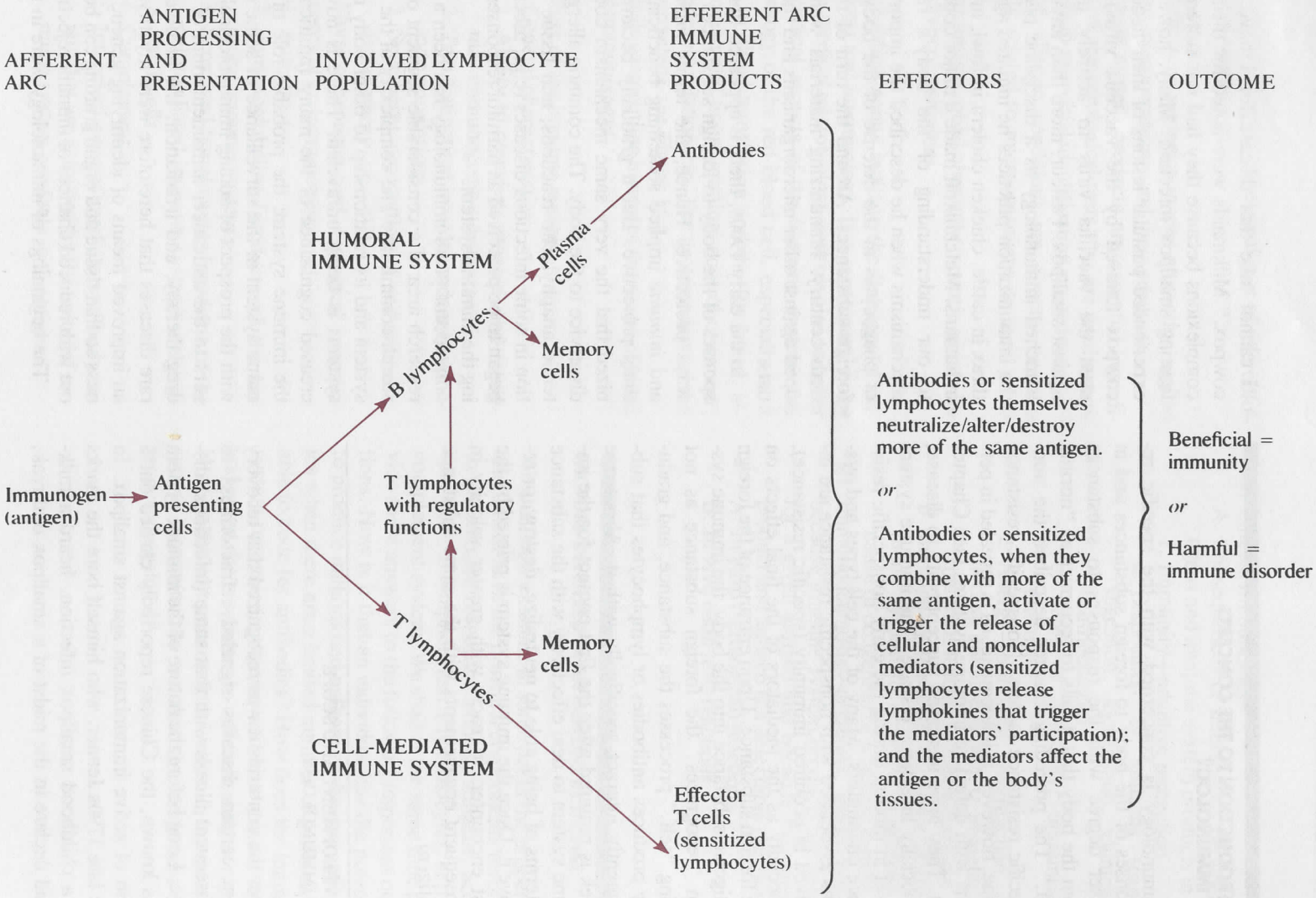
Over the centuries it was recognized that recovery from certain diseases signaled refractoriness to subsequent illness with that same (infectious) disease. Long before the nature of the immune system was known, the Chinese reportedly carried out a form of active immunization against smallpox. In the late 1790s Jenner, who himself bore the marks of a childhood smallpox infection, heard a milkmaid declare in the midst of a smallpox outbreak, "I cannot take that disease for I have had the cowpox." Milkmaids were noted for their smooth complexions because they had not suffered a disfiguring smallpox infection. Many, however, had experienced pustular lesions of their hands called cowpox (caused by the vaccinia virus). Jenner used the vaccinia virus to actively immunize against smallpox. Pasteur, more than anyone else, launched immunology as a discipline because of the immunization practices he initiated against anthrax in cattle, chicken cholera in fowl, and rabies in humans. Metchnikoff made a major contribution to our understanding of the body's resistance mechanisms when he described the important role of phagocytes in the defense of the body against foreign substances. Around the turn of the twentieth century, immunizing materials were developed against other microorganisms and their products.

In the early 1900s, then, it appeared that the responses of the body to foreign substances were always protective. Hence, the terms *immunology* and *immune* implied something beneficial, something protective. But it gradually became recognized that the very same mechanism could do a disservice to the body. The common allergies, systemic anaphylactic reactions, and tissue destruction in some infectious diseases (e.g., tuberculosis) began to be perceived as harmful responses involving the immune system.

In recent years immunology has been a favorite research area of a considerable segment of the research community. The complexity of the immune system and its relationship to other body response systems is being unraveled. There is now an increased cognizance of the many ramifications of the immune system: the probable role of the immune system in the surveillance of some cancers, with the prospect of adding immunological maneuvers to the anticancer armamentarium of surgery, drug therapy, and irradiation; the involvement in rare diseases that heretofore were mystifying; and an improved means of identifying genetic relatedness so that tissue and organ grafting can be carried out with reduced chances of immunorejection.

The beginnings of immunology were in microbi-

Fig. 15-1. Overview of the immune response.



ology. Immunology is an integral part of microbiology for these reasons:

1. The immune system provides protection against many microorganisms and their products.
2. Immune system products are essential ingredients of the serological tests that are used extensively in microbial identification procedures.
3. Some allergic conditions are directly or indirectly related to microorganisms or their products.
4. Some antimicrobial chemotherapeutic agents incite hypersensitivities.

The preceding points indicate the linkages of microbiology and immunology. It is customary, however, in medical microbiology courses also to cover other clinical aspects of the immune system: the immunology of the red blood cell, especially as it relates to blood transfusion and the Rh factor; allergy; organ and tissue grafting; control of cancer; fetal-maternal relationship. For an overview of the immune system, see Figure 15-1.

The following outline indicates the scope of basic medical immunology:

I. Nature of the immune system
  A. General characteristics
    1. Ability to perceive, process, and recognize immunogenic substances
    2. Two distinct but interdependent lymphocyte populations
    3. Specificity, recognition of self antigens, and memory
  B. Physicochemical and biological components of an immune response
    1. Antigens
    2. Immunoglobulins (products of the humoral immune system)
    3. Sensitized lymphocytes (products of the cell-mediated immune system) and regular T cells
    4. Complement
    5. Intermediates and mediators of body origin that are activated by the union of immune system products with antigens

II. Clinical Immunology
  A. Consequences of interactions between antigens and immune system products in vivo
    1. Immunity—adaptive protection
      a. Disposition of antigens (capture, digestion, localization, elimination, neutralization)
      b. States of immunity; immunization practices
    2. Allergy—primarily overreaction of a normal immune system to noninfectious materials leading to harmful effects
  B. Immune system defects; failure of the immune system to respond to antigens because of congenital or acquired defects

III. Immunodiagnosis—diagnosis made possible because immune system products are specific for a given antigen and because the union of product and antigen is a detectable reaction
  A. In vitro (serological) reactions
  B. In vivo (skin test) reactions

FACTORS INVOLVED IN AN IMMUNE RESPONSE

We shall now examine in some detail the properties and functions of the principal components involved in an immune response, namely, antigens, accessory cells, B lymphocytes, T lymphocytes, and the complement system. We shall at the same time provide some answers to the following important questions: How is it that an individual has the potential to produce millions and millions of diverse antibodies when there are a limited number of genes available to code for antibodies? How does the immune system recognize antigens, and how does it route and adapt the responses to different antigen types? How do the components involved in an immune response communicate so that the responses are properly regulated?

ANTIGENS

Immunogenicity and Reactivity

An antigen is a substance with certain properties that is recognized by the immune system as being foreign or not normal self. In the latter instance it sometimes happens that the immune system reacts against the body's own cells or molecules to lead to an autoimmune disease (as described in Chapter 17). An antigen stimulates the immune system to produce antibodies or sensitizied T cells, and later the antigen specifically reacts with the antibodies or T cells. The preceding statement implies two features of antigens—immunogenicity and reactivity. *Immunogenicity* refers to the quality of being able to stimulate an immune response. *Reactivity* refers to the capacity to combine with the *formed* immune products, namely with the antibodies or with the sensitized T cells. The term *immunogen* is sometimes used in place of the term *antigen* in contexts in which the reference is to the stimulatory property of an antigen. A vaccine, for example, is properly called an immunogen.

Typical Antigens

Typical foreign antigens that the immune system responds to include the various types of infectious disease agents and their products (e.g., toxins), proteins of foreign serum, cells of the host that harbor infectious agents, mismatched transfused red blood cells, transplanted tissues, capsular and other polysaccharides, and vaccines.

Physical Makeup: Particulate and Soluble Antigens

On the basis of their physical makeup antigens are particulate or soluble. Particulate antigens are particle-sized, as exemplified by intact bacterial cells and red blood cells. Soluble antigens are molecular in size, as exemplified by the various proteins of serum and bacterial toxins. What are frequently called antigens, for example, bacterial cells, in actuality are a composite of many diverse antigenic molecules. The various structural and molecular components of a bacterial cell—capsules, flagella, pili, lipopolysaccharide, teichoic acid, and subfractions thereof—all are antigenic. Disassembly of the native antigen such as a bacterial cell occurs in vivo during the efferent phase of the immune response.

Attributes of Antigens

The ability of a substance to elicit an immune response and the type and level of that response are related to such characteristics as chemical composition, molecular size, molecular complexity, digestibility of the native (original or unprocessed) antigen, foreignness, and accessibility of determinant groups (see next paragraph) to lymphocyte receptors. Natural proteins and many polysaccharides are highly antigenic, nucleic acids are less potent antigens, and pure lipids are weakly antigenic or nonantigenic.

Antigenic Determinants

Antigenic molecules are composed of a carrier portion and antigenic determinants. The bulk of most antigenic molecules is the inner, central carrier portion against which the immune system ordinarily does not form antibodies or sensitized lymphocytes. On the surface of the antigenic molecule are small, discrete chemical groups called antigenic determinants (or antigenic sites) whose chemical and physical nature confers specific characteristics on the antigen molecule and against which the immune system forms antibodies or sensitized lymphocytes. It is the antigenic determinant that fits into the antigen-binding site of immunoglobulins. The number on any one antigen and the chemical and physical types of determinants can vary considerably. Some determinants are more immunogenic (antibody inducing) than others, and therefore the antibodies produced against them are the dominant antibodies in the serum (see the discussion of monoclonal antibodies in the next chapter). Such determinants, sometimes referred to as *epitopes*, are said to be immunodominant.

Haptens

Certain molecules or substances cannot by themselves induce antibody formation. This inability is usually due to their small size. When coupled with carrier molecules, however (and coupling can be

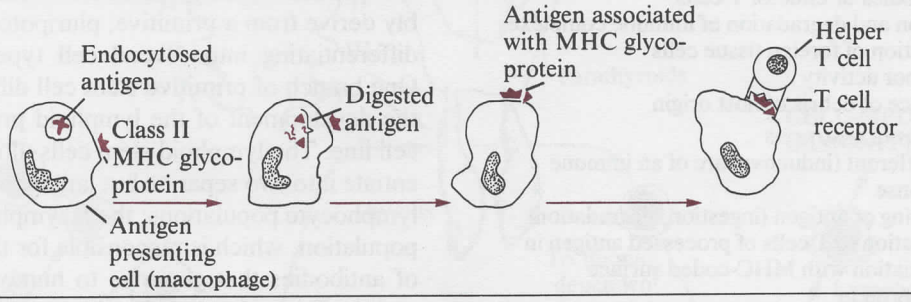


Fig. 15-2. Processing and presentation of antigen by an antigen-presenting cell. Antigens taken up by macrophages undergo a series of changes the exact mechanisms for which remain unknown. The immunogenic fragments become associated with the cell surface glycoproteins (also referred to as Ia antigens) that are encoded by the major histocompatibility complex (*MHC*). Helper T cells that have the appropriate antigen receptor molecules are activated by the antigen–MHC glycoprotein complex.

achieved artificially in the laboratory or can occur naturally), small molecules can serve as antigenic determinants. In this capacity they are called *haptens*. They are said to be *partial* or *incomplete antigens*. They interact specifically with homologous antibodies. Haptens do not incite antibody production unless coupled with a carrier protein. As will be described later, many of the naturally occurring allergenic substances of allergic contact dermatitis are haptens.

Thymus-dependent and Thymus-independent Antigens

The majority of natural antigens do not elicit a response by the antibody-producing B cells unless helper T cells also participate. Such antigens are therefore termed *thymus-dependent antigens* because the helper T cells have to pass through the thymus gland to gain competence. Some antigens, however, can induce antibody synthesis without the cooperation of helper T cells. These antigens are termed thymus-independent (TI) antigens. TI antigens generally are high molecular weight polymers, usually polysaccharides, with an array of rather simple repeating antigenic determinants.

Antigen Processing, Modification, and Presentation by Accessory Cells

Many antigens before they can stimulate an immune response must first be processed and then "presented" by accessory cells to lymphocytes (Fig. 15-2). Macrophages (Table 15-1) and dendritic cells (reticuloendothelial system cells) perform this accessory role. The precise manner in which accessory cells handle the antigen remains to be elucidated. Macrophages probably engulf large, complex antigens, partially degrade them, possibly modify them, and then present the processed antigen on their surfaces. Intracellular processing, however, is probably not obligatory for small antigens such as peptides, which merely need to adhere to the surfaces of the accessory cells.

GENETIC RESTRICTIONS IN ANTIGEN RECOGNITION. There are some genetic restrictions on the interactions between antigen-receptive T cells (helper T cells) and antigen-presenting cells. Effective interaction occurs only when the two cells share certain host-determined glycoproteins on their cell membranes. The glycoproteins are coded for by genes on the major histocompatibility complex (MHC) region of chromosome 6 in humans. The MHC will be explained more fully in a later section. Briefly, the restriction is that the helper T cell will recognize the antigen only if the antigen is presented in association with a self MHC antigen on the surface of an accessory cell.

MACROPHAGE INTERLEUKIN-1 AS AMPLIFIER. Antigen-presenting macrophages also release an amplifying

Table 15-1. Some Functions of Macrophages (Mononuclear Phagocytes)

ROLES IN RESISTANCE AND SCAVENGING
Ingestion and destruction of microorganisms and foreign particles without the involvement of antibodies or effector T cells
Ingestion and degradation of immune complexes
Elimination of foreign tissue cells
Antitumor activity
Clearance of debris of host origin
ROLES IN IMMUNE REACTIONS
Roles in afferent (inductive) arc of an immune response
Processing of antigen (ingestion, degradation)
Presentation to T cells of processed antigen in association with MHC-coded surface glycoprotein
Secretion of interleukin-1 (activates T cells and stimulates T cell proliferation)
Roles in efferent (effector) arc of an immune response
Destruction of antigens through mechanisms involving
Opsonins—Fc portion of an immunoglobulin and complement fragment C3b bind antigens to macrophages as a prelude to ingestion and destruction
Lymphokines—T cell (primarily) products that promote the attraction, activation, and proliferation of macrophages at the site of antigen incursion

MHC = major histocompatibility complex; Fc = crystallizable fragment.

factor, *interleukin-1*, at the time of antigen presentations. Interleukin-1 causes antigen-stimulated lymphocytes to produce T cell growth factor (called *interleukin-2*), which in turn leads to the activation and proliferation of reactive T cells.

B LYMPHOCYTES

Ontogeny of Two Major Lymphocyte Populations—B Lymphocytes and T Lymphocytes

Hematopoietic (blood cell–forming) stem cells reside in the yolk and the liver in embryonic life. Postnatally the stem cells are primarily located in the bone marrow, the principal site of hematopoiesis (Fig. 15-3). Continuous formation and development of red blood cells, granulocytes, monocytes, megakaryocytes (source of platelets or thrombocytes), and lymphocytes take place in the bone marrow. The different blood cell types probably derive from a primitive, pluripotent (capable of differentiating into several cell types) stem cell. One branch of primitive stem cell differentiation is the development of the lymphoid progenitor stem cell line. The lymphoid stem cells ultimately differentiate into two separate but largely interdependent lymphocyte populations: the B lymphocyte (B cell) population, which is responsible for the production of antibodies that give rise to humoral immunity; and the T lymphocyte (T cell) population, which is responsible for the production of sensitized lymphocytes that give rise to cell-mediated immunity. The T lymphocyte population also has the major immunoregulatory role. The B cell and T cell populations differ from each other developmentally, antigenically, functionally, and at given times morphologically and in distribution. The differentiation and maturation of each population occur in several lymphoid compartments, namely the central or primary lymphoid organs (bone marrow for B cells, thymus for T cells), and the peripheral or secondary lymphoid organs (different areas primarily of the lymph nodes and spleen).

Differentiation and Maturation of B Cells

A central lymphoid organ for B cells was first discovered in chickens. It is the cloaca-associated organ known as the bursa of Fabricius—hence the term *B cell*. The bone marrow of mammals is regarded as the principal central lymphoid organ for B cells. The peripheral or secondary lymphoid tissues where antibody formation occurs are the lymph node, red pulp of spleen, bone marrow, and lamina propria of secretory glands. During differentiation and maturation (Fig. 15-4), a B cell undergoes a succession of changes in location, morphology, immunocompetence, and function. Pre–B cells are immunoincompetent and have intracytoplasmic IgM (the heavy polypeptide chain part of

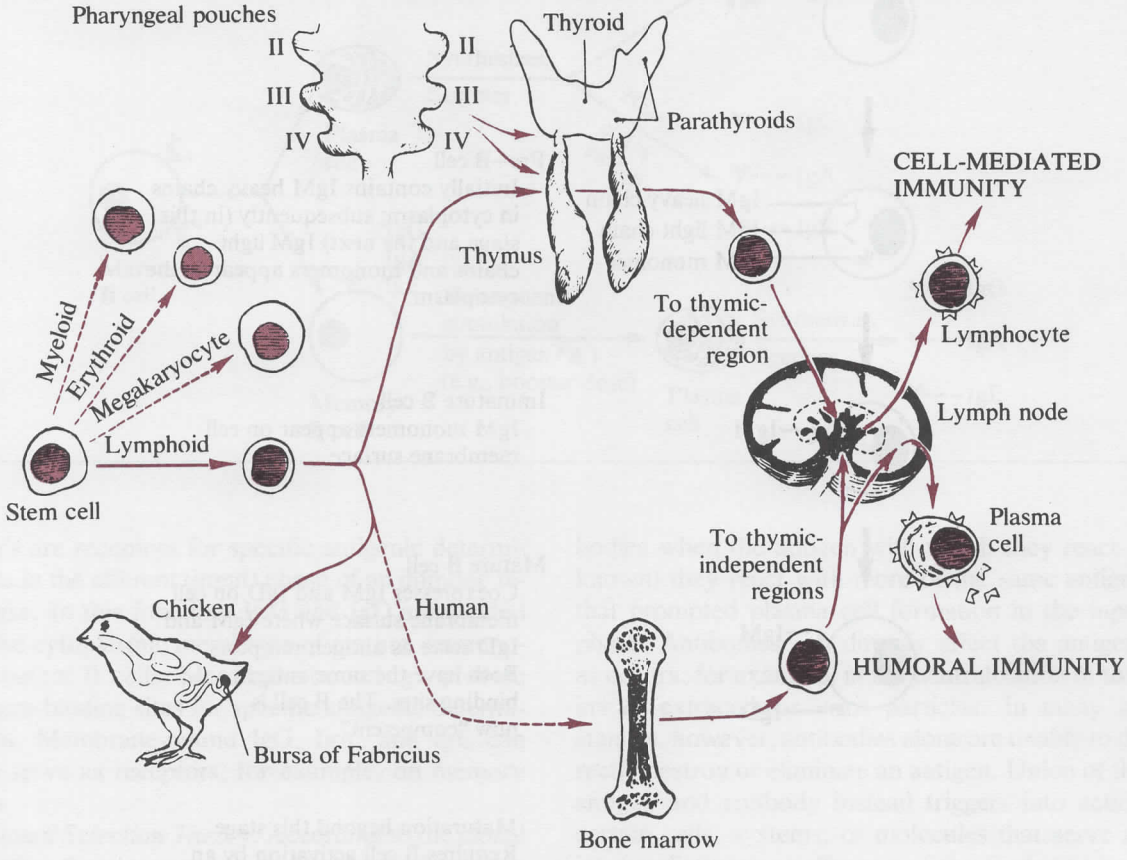


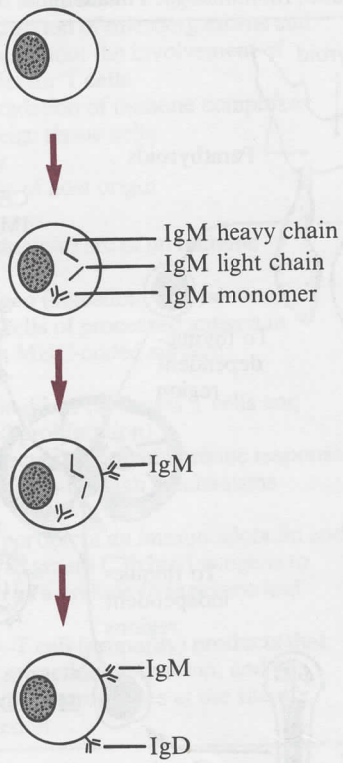
Fig. 15-3. Development of the immune response. Stem cells develop into blood cell–forming progenitor cells (myeloid, erythroid, megakaryocyte) and into lymphoid cells. The lymphoid cells eventually develop into T lymphocytes or B lymphocytes. (Adapted from J. A. Bellanti, *Immunology*. Philadelphia: Saunders, 1978.)

the antibody molecule); immature B cells are immuno*in*competent and have cell surface IgM; mature B cells are immunocompetent and have cell surface IgM and IgD; transient cell types called lymphoblasts are proliferative (multiplying) cells that convert into the most differentiated state of B cells, namely the plasma cell (*plasma cell* does not mean a cell of the blood fluid plasma but is the name of a cell type that has a distinctive microscopical morphology). It is the plasma cells that secrete antibodies, which are the effectors of humoral immunity (Fig. 15-5). Not all of the lymphoblast cells become plasma cells. Some differentiate into memory cells.

Immunoglobulins (Antibodies)

FIVE CLASSES OF IMMUNOGLOBULINS. Immunoglobulins are glycoproteins that are responsible for humoral immunity, which is also called antibody-mediated immunity. There are five immunoglobulin classes, IgM, IgG, IgA, IgE, and IgD, the basis of the classification being differences in the composition of the heavy chains (H chains) of the Ig mole-

Fig. 15-4. Stages of B cell differentiation.



Lymphoid stem cell

Pre—B cell
Initially contains IgM heavy chains in cytoplasm; subsequently (in this stage and the next) IgM light chains and monomers appear in the cytoplasm.

Immature B cell
IgM monomers appear on cell membrane surface.

Mature B cell
Coexpresses IgM and IgD on cell membrane surface where IgM and IgD serve as antigen receptors. Both have the same antigen binding sites. The B cell is now "competent".

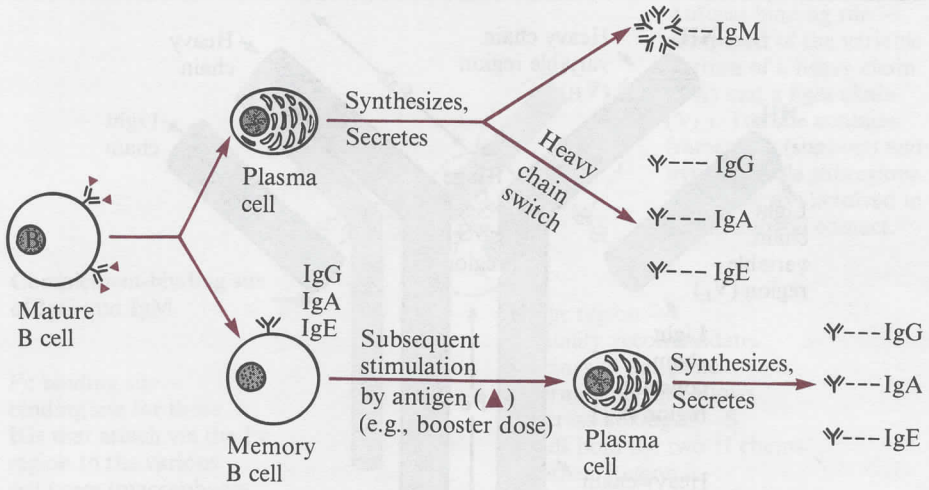
Maturation beyond this stage
Requires B cell activation by an appropriate combination of antigen, helper T cells and macrophage cells ("antigen driven") or by agents that induce mitosis.

cule. The classes differ in respect to biological activities, as will be discussed later.

Isotypes. The Ig classes differ antigenically. This means that when the Ig classes are injected into laboratory animals as antigens, the animals produce antibodies that are specific for each of the Ig types. Antigenic substances that are variants of the same antigen (as Ig classes are) and that appear in the same species are called *isotypes*. Accordingly the five Ig classes as antigens are said to be isotypes.

MEMBRANE-BOUND IMMUNOGLOBULINS AS ANTIGEN RECEPTORS AND SECRETED IMMUNOGLOBULINS AS EFFECTORS OF HUMORAL IMMUNITY. Two roles that Ig's fulfill are as receptors and as effectors.

Fig. 15-5. Differentiation of mature B cell upon antigen stimulation.

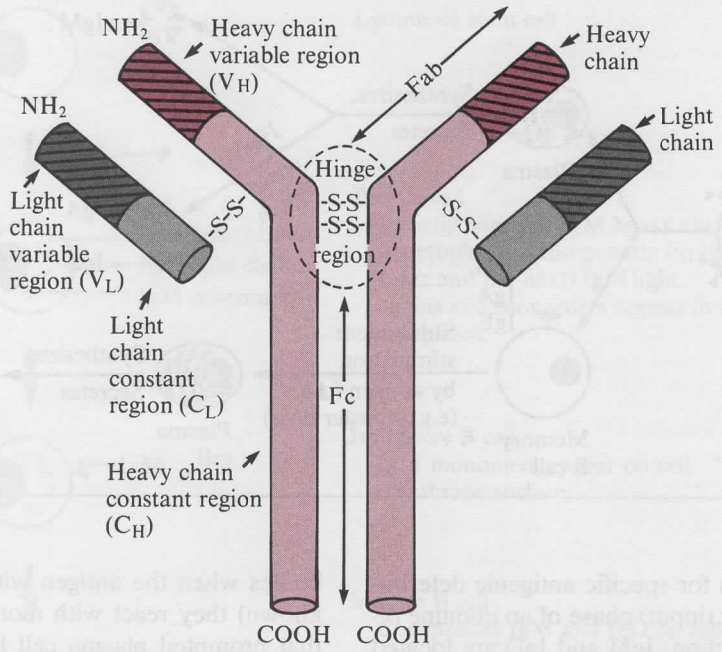


Ig's are receptors for specific antigenic determinants in the afferent (input) phase of an immune response. In this function, IgM and IgD are located on the cytoplasmic membrane of mature, immunocompetent B cells. Membrane-bound Ig's provide antigen-binding sites for specific antigenic determinants. Membrane-bound IgG, IgA, and IgE can also serve as receptors, for example, on memory cells.

Clonal Selection Theory. According to the clonal selection theory proposed by Sir MacFarlane Burnet in the 1950s there is a genetically determined, fixed repertoire of antigen-binding sites in the total immune lymphocyte population. Incoming antigen selectively stimulates only those lymphocytes that exhibit the preordained complementary antigen-specific receptors. The lymphocyte, upon binding the antigen, is activated and proliferates to form a clone of antibody-secreting plasma cells. The number of clones formed is theoretically identical to the number of antigenic determinants on the antigen.

The second role of immunoglobulins is that they are effectors of immunity in the efferent (output) phase of the immune response when as secreted immunoglobulins (which can now be called antibodies when the antigen with which they react is known) they react with more of the same antigen that prompted plasma cell formation in the input phase. Antibodies can directly affect the antigen, as occurs, for example, in the neutralization of toxins or extracellular virus particles. In many instances, however, antibodies alone are unable to directly destroy or eliminate an antigen. Union of the antigen and antibody instead triggers into action certain cells, systems, or molecules that serve as intermediates or as effectors of the final action on the antigen. Antibacterial antibodies known as opsonins, for example, attach by one end of the Ig molecule (Fab end) to the bacterial cell and with the other end (Fc end) to a phagocyte, which facilitates ingestion of the bacterial cell by the phagocyte. The phagocyte exercises the final effect on the bacterium (the antigen) by digesting it. Another example, an antibody called a bacteriolysin, does not itself lyse the bacterial cell. The union of the antibody and the bacterial cell activates the complement system, and it is the membrane attack unit of the complement system that leads to the lysis of the bacterial cell (see Chap. 15, The Complement System).

Fig. 15-6. Basic structure of immunoglobulin. (S–S = disulfide bonds; Fab = antigen-binding fragment; Fc = crystallizable fragment; NH_2 = amino terminal; COOH = carboxy terminal.)



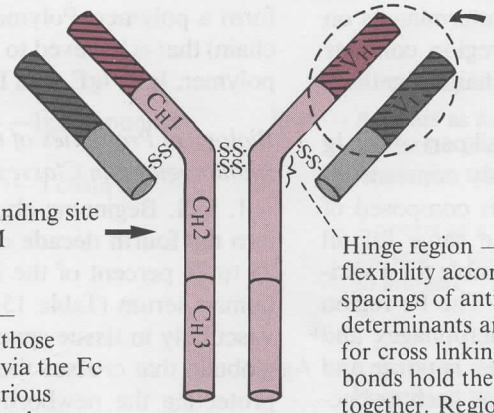
Over the years antibodies have been referred to by names that are descriptive of their in vitro and in vivo reactions with their homologous antigens. Thus antibodies have been called *precipitins, agglutinins, cytolysins, antitoxins, opsonins*, and *neutralizing antibodies* (the actions of the antibody types are explained in Chapter 16).

Structure of the Basic Immunoglobulin Molecule

HEAVY CHAINS AND LIGHT CHAINS. Immunoglobulins have a basic pattern of four polypeptide chains (Fig. 15-6). Two of the chains are of a higher molecular weight and are called the *Heavy (H) chains*. They are identical. Differences in H chain composition determine the five Ig classes. The two *light (L) chains* have a lower molecular weight and are approximately one-half the length of the H chains. L chains are of two types, kappa or lambda. The kappa is always paired with an identical kappa in a given Ig molecule, and a lambda with another lambda. Each L chain is linked to an H chain by disulfide bonds. Two such identical halves are joined together by disulfide bonds and by noncovalent forces to form the basic four-chain polypeptide unit. A schematic representation of the Ig molecule depicts it as a Y-shaped structure. The terminal of each leg of the Y (called the *N-terminal*) has a variable amino acid sequence and is called the *Fab* (from *fragment, antigen-binding*). The tail part of the Y has a constant (C) amino acid sequence, and its terminal is referred as the *C-terminal* or *carboxy terminal*. The tail part is called the *Fc* (from *fragment, crystallizable*).

DOMAINS. Each H and L chain consists of several repeating loops that are held together by intrachain disulfide bonds. Each loop comprises a domain. L chains have one variable (V_L) domain and one con-

Fig. 15-7. Major functional regions of the immunoglobulin molecule.



stant (C_L) domain. The H chains of IgG and IgA have one variable domain (V_H) and three constant domains, C_H1, C_H2, C_H3. IgM and IgE have an additional H chain domain, C_H4.

VARIABLE REGION OF THE FAB PORTION. The variable regions of the H and L chains are across from each other and form an antigen-binding (Fab) site (Fig. 15-7). The variableness is due to differences in amino acid composition and sequences. The variable regions have two principal subregions: the hypervariable subregions and the framework subregions. The hypervariable regions (also referred to as complementarity-determining regions or as "hot spots") are the areas actually involved in antigen binding. There are three such subregions on V_L and four on V_H. The hypervariable subregions comprise the idiotype of an Ig molecule (to be discussed in a later section).

The flexible framework regions flank the hypervariable regions and mold the V_H and V_L into a three-dimensional cavity-like structure that accommodates an antigenic determinant that comprises no more than five to seven amino acids or glucoses. The simplest antibody molecule has an identical antigen-binding site at each leg of the Y, making the molecule divalent.

HINGE REGION. The H chains have a flexible hinge region between the C_H1 and C_H2 domains where the two arms of the Y join the tail part of the Ig molecule. This region demarcates the Fc from the

Fab when the Ig molecule is digested with the enzyme papain. This region, because it swings through a 30-degree arc, allows the distance between the two antigen-binding sites to vary, thereby improving efficiency of antigen binding and of cross linking (binding antigenic determinants on neighboring antigens). The hinge region contains disulfide bonds that join the two H chains together.

CONSTANT REGION (FC REGION). The tail part of the Ig molecule, the part that contains only constant regions of the paired heavy chains, is composed of amino acid sequences that are the same for all chains of the same type. It is responsible for a variety of effector biological functions. The Fc region binds to specific receptors on macrophages and neutrophils, thereby facilitating the capture and ingestion of antibody-coated antigens such as bacteria. The Fc region may also bind to other cell types such as mast cells and eosinophils. Antibodies that are bound to cells are said to be *cytophilic* or *cytotropic*. Different cells (neutrophils, macrophages, mast cells) express receptors for the Fc of different Ig classes. The Fc region of IgG and IgM also binds and thereby activates the first component of the complement system (which is described later in this chapter). Maternal IgG antibodies are the only antibodies that cross the placenta into the fetus, and that passage is mediated by the Fc portion of the IgG molecule.

Heavy Chain Switch. Let us return momentarily to the mature B cell stage in which there is membrane-bound IgM and IgD and the cell is immunocompetent, ready to react with its specific antigenic determinant. The membrane-bound IgM and IgD both have the same antigen specificities, that is, the same variable region sequences on the Fab part of the Ig molecule, and all the Igs to be produced by the cell and its clonal progeny will have the same antigen-binding specificity. The heavy chain part of the secreted Ig, however, can remain IgM or it can switch to IgG, IgA or IgE. The fate of the IgD is unknown.

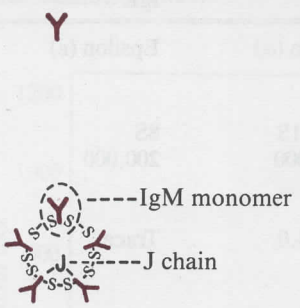
IMMUNOGLOBULIN POLYMERS. IgA can exist as a monomer (a single structural unit of two H and two L chains), dimer, or trimer (Fig. 15-8). IgM is a monomer when it is serving as a receptor Ig on the B cell surface and is a pentamer as secreted Ig. Dimers, trimers, and pentamers are respectively two, three, or five basic Ig molecules joined together to form a polymer. Polymers have a J chain (joining chain) that is believed to initiate the assembly of the polymer. IgG, IgE, and IgD do not polymerize.

Biological Properties of the Five Immunoglobulin Classes

1. IgG. Beginning about the age of 2 years and into the fourth decade of life IgG makes up about 75 to 80 percent of the immunoglobulin in normal human serum (Table 15-2). It is also found extravascularly in tissue spaces. It is the only immunoglobulin that crosses the placental barrier, thereby protecting the newborn child temporarily against certain infectious diseases that the mother has antibodies against (Fig. 15-9). Functionally, IgG neutralizes toxins, inactivates extracellular viruses and bacteria, and prepares them for phagocytosis in the process known as *opsonization*. IgG serves as the blocking antibody in hyposensitization of atopic allergies (see under Atopy, in Chapter 17). When combined in certain proportions with antigen and complement factors, it forms complexes (antigen-antibody-complement) that are harmful (see under Arthus Reaction, Serum Sickness, and Immune [Toxic] Complex Disease, in Chapter 17) (see Fig. 17-5).

2. IgM. IgM is the largest of the immunoglobulin molecules, the letter *M* in fact being derived from *macroglobulin*, which means "large globulin." Secreted IgM is composed of five of the basic four-chain units that are attached to each other by disulfide bridges at their Fc portion. IgM is found primarily intravascularly, where it constitutes about 7 to 10 percent of the immunoglobulin in normal adult serum. The IgM pentamer (having five units) has ten antigen-determinant binding sites. IgM antibodies are the first to be synthesized in life. The human fetus is capable of forming IgM. In fact, its presence is indicative of fetal infection or exposure to antigens. IgM is the first class of antibody synthesized by adults in response to an antigen. Func-

Fig. 15-8. Structures of the immunoglobulin classes.



IgG — A monomer (single unrepeating unit). Most common antibody in serum.

IgM — Appears as a monomer on surface of immature B cells where it serves as antigen receptor.

— Appears as a pentamer in serum. Earliest Ig formed in response to antigen.

IgA — Appears as a monomer in serum. Formed in spleen and lymph nodes.

— Appears as a dimer and trimer on mucosal surfaces. Produced as a dimer with J chain in lamina propria of gut, lung, and glands. Secretory piece added upon transport across epithelial cells.

IgD — A monomer. Appears on surface of immature B cells where it expresses the same antigen receptor as IgM.

IgE — A monomer. Attaches to mast cells. Serves as the reaginic antibody of human anaphylaxis.

tionally, IgM antibodies are similar to IgG antibodies. They protect against extracellular antigens, and they form harmful complexes. Most of the naturally occurring antibodies (i.e., those that develop in the absence of known antigenic stimulation) belong to the IgM class. The cold agglutinins (react only at temperatures below body temperature) and ABO red blood cell agglutinins are in this latter category.

3. IgA. Although a considerable percentage (about 40 percent) of IgA appears intravascularly, its greatest importance derives from its presence on the epithelial surfaces in external secretions. It is found in saliva, tears, colostrum (milky fluid secreted by the mammary gland around the time of birth), and secretions on the surfaces of the intestinal, respiratory, and genital tracts. It is called *secretory IgA* (*sIgA*) when it appears in the exocrine

Table 15-2. Selected Physicochemical and Biological Properties of Human Immunoglobulins

Property	IgG	IgM	IgA	IgE	IgD
H CHAIN CLASS	Gamma (γ)	Mu (μ)	Alpha (α)	Epsilon (ε)	Delta (δ)
PHYSICOCHEMICAL PROPERTIES					
Sedimentation	7S	19S	7S–11S	8S	7S
Molecular weight (daltons)	148,000	900,000	170,000	200,000	180,000
Serum concentration (mg/ml)	8–16	0.4–2.0	1.4–4.0	Trace	0.03
Half-life in serum (days)	18–23	5	6	1.5	3
Number of four-chain units	1	5	1–3	1	1
Antigen-binding sites	2	10	2–6	2	2
BIOLOGICAL PROPERTIES					
Types of antibody	Agglutinin Precipitin Opsonin Neutralizing	Agglutinin Precipitin Opsonin Neutralizing	Secretory antibody on mucous surfaces	Reagin; skin-sensitizing	?
Fixes complement	Yes	Yes	No*	No*	No
Crosses placenta	Yes	No	No	No	No
Other distinct properties	Blocking antibody of atopic allergy hyposensitization	First Ig formed; ABO antibodies; receptor on B cell membrane	Found in serum and external secretions; provides local, topical immunity	Reaginic antibody of anaphylaxis and atopic allergies; attaches to mast cells	Receptor on B cell membrane

*Activates complement through the alternative pathway.

secretions. sIgA as a dimer has four components: two IgA molecules, a polypeptide J chain, and a polypeptide transport piece called *secretory component* or secretory piece. Two cell types are involved in the formation of sIgA (Fig. 15-10). The dimer, consisting of two molecules of IgA and the J chain, is primarily produced by submucosal plasma cells. The transport piece is added to the dimer in glandular epithelial cells before the dimer exits onto the mucosal surface. The epithelial cell, through which the dimer passes, synthesizes the secretory component, which becomes located across the epithelial cell membrane (transmembrane glycoprotein). The secretory component first serves as a receptor for IgA from the blood and is then picked up by the dimer. This newly formed complex is transported through the epithelial cell cytoplasm and secreted through the opposite side

Fig. 15-9. Probable IgG levels in the fetus and newborn. (From M. R. Allansmith. In F. Falkner [Ed.], *Human Development*. Philadelphia: Saunders, 1966.)

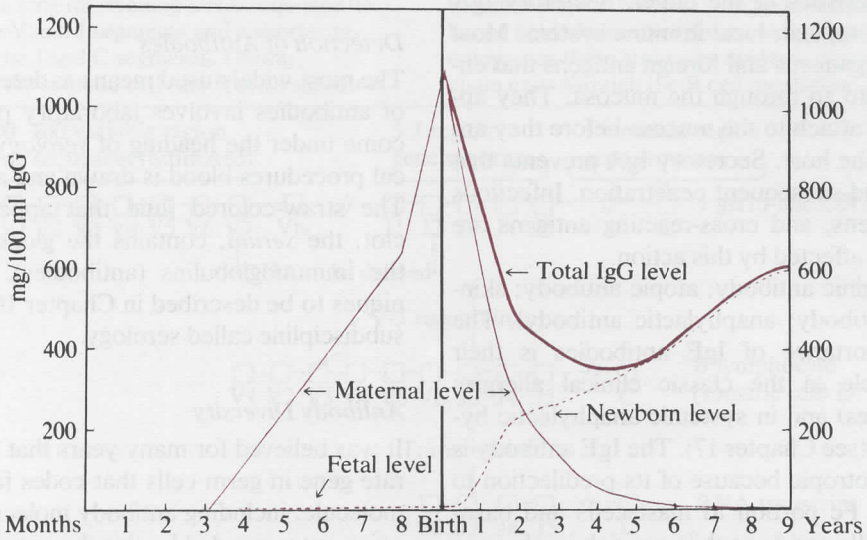
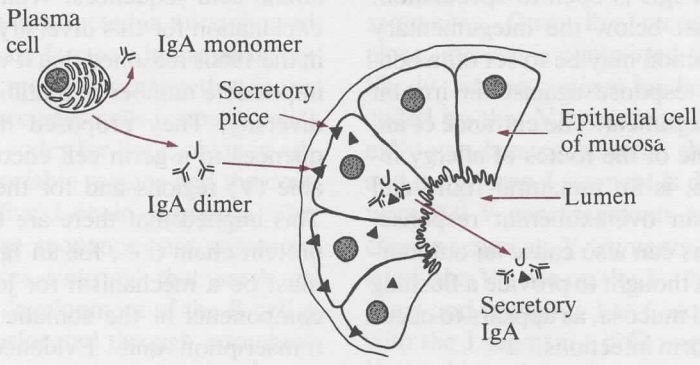


Fig. 15-10. IgA dimers (two units of IgA and a J chain) are formed in submucosal plasma cells. The secretory piece initially is located across the cell membrane (the piece is termed a *transmembrane glycoprotein*) of mucosal epithelial cells where it serves as a receptor for the IgA dimer. The IgA dimer–secretory piece complex is transported through the cytoplasm of the epithelial cell and secreted through the opposite side of the cell. The secreted IgA provides local, topical immunity on the mucosal surface.



of the cell where it exits into the lumen of the gland or onto the mucosal surface.

Secretory IgA is resistant to enzymatic digestion, evidently because of the molecular conformational characteristics of the dimer. Secretory IgA functions as a topical, local immune system. Most of the microorganisms and foreign antigens that enter the body do so through the mucosa. They apparently must attach to the mucosa before they are able to harm the host. Secretory IgA prevents that attachment and subsequent penetration. Infectious agents, allergens, and cross-reacting antigens are believed to be affected by this action.

4. IgE (reaginic antibody; atopic antibody; skin-sensitizing antibody; anaphylactic antibody). The principal importance of IgE antibodies is their pathogenic role in the classic clinical allergies (atopic allergies) and in systemic anaphylactic hypersensitivity (see Chapter 17). The IgE antibody is said to be cytotropic because of its predilection to attach via its Fc portion to mast cells and basophils. Mast cells and basophils are rich in pharmacologically active substances, of which histamine is the most important. When antigen unites with the attached IgE molecule in a sensitized individual, the combination on the mast cell surface triggers the release of histamine and other potent substances. The released substances go on to exert a number of effects that may be deleterious (see the discussion of systemic anaphylaxis in Chapter 17). The protective role of IgE is open to speculation. IgE acts primarily just below the integumentary surface, where its function may be to set off a beneficial inflammatory response against an irritant that penetrates the integument. The entrance of antigens by injection, one of the routes of allergy induction and response, is an unnatural route and sometimes leads to an overexuberant response. IgE-mediated reactions can also cause an outpouring of mucus, which is thought to provide a flushing action on the intestinal mucosa, as appears to occur in certain intestinal worm infections.

5. IgD is found only in very small amounts in the serum, but it occurs on the surface of mature immunocompetent B lymphocytes. On the lymphocyte surface it serves as a specific membrane receptor for its "intended" antigenic determinants. The membrane-bound immunoglobulin is produced within the lymphocyte on whose surface it resides rather than being absorbed from the serum.

Detection of Antibodies

The most widely used means to detect the presence of antibodies involves laboratory procedures that come under the heading of *serology*. For serological procedures blood is drawn and allowed to clot. The straw-colored fluid that appears above the clot, the *serum*, contains the globulins, including the immunoglobulins (antibodies). Various techniques to be described in Chapter 16 constitute the subdiscipline called serology.

Antibody Diversity

It was believed for many years that there is a separate gene in germ cells that codes for each protein molecule, including antibody molecules. Immunologists were puzzled by this, however, because they knew that there were many millions to billions of antibodies with different specificities yet there were no more than an estimated total of one million genes in animals that coded for all the proteins of the body, not just for the Igs. It was furthermore known that the amino acid sequences in the constant part of each Ig class were identical, whereas the variable part of the molecule had differing amino acid sequences. What could be the genetic explanation for this diversity? Dreyer and Bennett in the 1960s reasoned that it would have required an impossible number of mutations to account for the diversity. They proposed that separate gene sequences in a germ cell encoded for multiple variable (V) regions and for the constant (C) region. This implied that there are two genes for a single protein chain (i.e., for an Ig chain) and that there must be a mechanism for joining the two genetic components in the somatic cell to form a single transcription unit. Evidence obtained in animal studies using modern DNA manipulation methods of restriction eudonucleases, recombinant DNA, cloning, and rapid DNA sequencing proved the proposal correct.

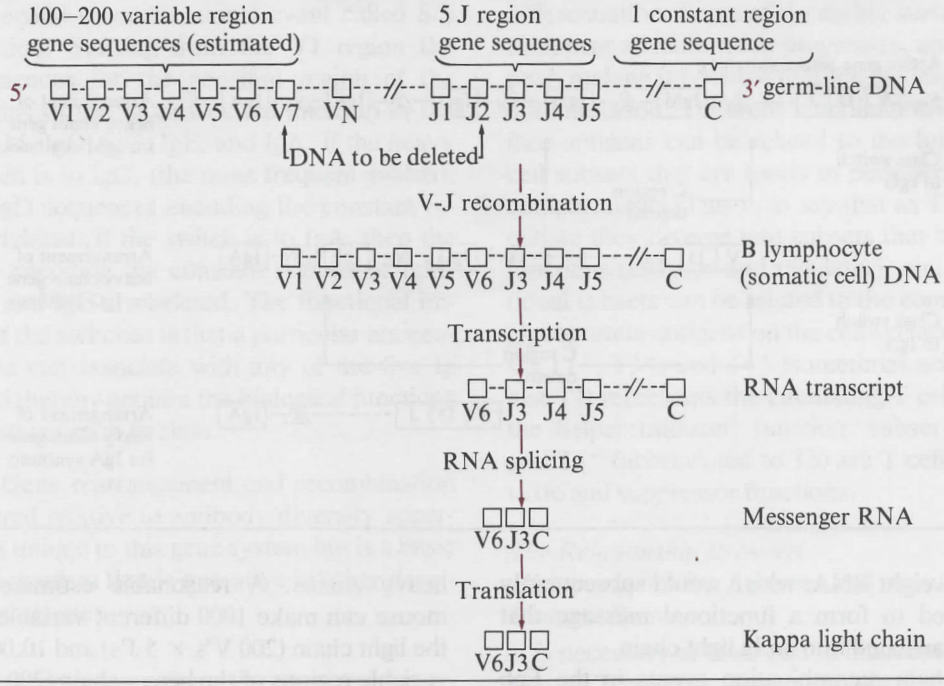
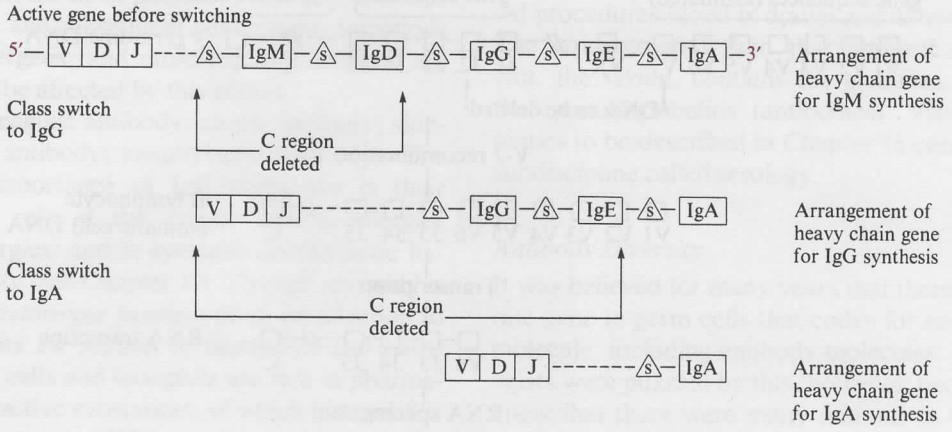


Fig. 15-11. The generation of antibody diversity in a kappa light chain (human). Germ-line DNA for the light chain contains multiple variable (V) and joining (J) gene sequences and a single constant (C) region gene sequence. There is a long intervening DNA sequence (intron) between the V and J segments and a shorter sequence between the J and C segments. During recombination in the somatic cell (the B cell), segments of DNA are deleted as the chosen V gene sequence comes to be translocated next to one of the J segments. The entire gene is transcribed. The intervening sequences and the extra J's then are spliced out to yield the messenger RNA that is to be translated into the kappa light chain. (Not illustrated: A leader segment accompanies these steps; the leader is cleaved away as the chain exits through the B cell membrane.)

The studies originally conducted in mice showed that the V and C genes are far apart in the germ line (embryonic DNA) and move closer together in the DNA of lymphocyte precursor cells (somatic cells) (Fig. 15-11). DNA that codes for the L chain in addition to the multiple variable regions and the constant region contains five J chain segments. The V, J, and C region gene sequences are separated by noncoding sequences (introns) that serve as "spacers." During the development of the B cell a V gene segment is translocated through intrachromosomal recombination to a site next to a J segment, thereby forming a contiguous J sequence with intervening introns. Any of the multiple V gene segments can be joined to any of the J gene sequences. Once this recombination has taken place, the cell is committed to synthesizing an antibody whose antigen-binding specificity is encoded by that VJ sequence. The DNA that originally was located between the chosen V segment and the chosen J segment is deleted. Thus, if there were 200 V gene segments and the sixth was the chosen segment, V segments 7 to 200 would be deleted (the V's are on the 5′, "upstream" end of the gene) and segments 1 to 6 would remain. Likewise with the J segment—if J3 were chosen then J1 and J2 would be deleted and J3, J4, and J5 would remain. The resulting gene, which would contain V segments 1 to 6, J segments 3 to 5, introns, and the constant segment, would be transcribed into high

Fig. 15-12. Heavy chain switch (S/S recombination). The recombinational steps on the 5′ (left) side of the active gene that produced the V, D, and J sequence (the variable region) are shown in Fig. 15-11. The D (diversity) gene segment is found only in heavy chains. The constant (C) region gene sequences are lined up in the order of IgM, IgD, IgG, IgE, and IgA. Each constant region gene is preceded by a switching signal (*S*). This arrangement leads to IgM synthesis if there is no gene switching. The switch to IgG secretion involves deletion of the S, IgM, S, IgD sequence and the translocation of the VDJ transcription unit to a position next to the IgG gene sequence. The switch to IgA secretion involves deletion of the S, IgM, S, IgD, S, IgG, S, IgE sequence and the translocation of VDJ to a position next to the IgA sequence.



molecular weight RNA, which would subsequently be processed to form a functional message that would be translated into an Ig light chain.

Heavy chain recombination events in the Fab portion of the Ig molecule are like those just described for the light chain, but with one difference. The variable region of the heavy chain has an additional gene segment called the D (diversity) gene segment, and V and J recombinations with alternative sequences in this segment afford additional chances for variability. There are at least 10 of these D segments in mice.

This capability of recombining selected sequences of germ-line DNA into a single active sequence in somatic cells explains how a large repertoire of antibody genes can be generated without having to have a germ-line gene for each antibody specificity. Gene-counting experiments in mice indicate that there are several hundred V gene segments for light chains and a similar number for heavy chains. A reasonable estimate is that a mouse can make 1000 different variable regions of the light chain (200 V's × 5 J's) and 10,000 different variable regions of the heavy chain (200 V's × 5 J's × 10 D's). The combination of the variable regions of the L and H chains to form an antigen-binding site thus creates a potential 10,000,000 antibody specificities.

HEAVY CHAIN SWITCH DURING ANTIGEN-DRIVEN MATURATION OF B CELLS: S/S RECOMBINATION. A type of diversity that involves the biological functions of the antibody molecule occurs at the other end of the heavy chain through a mechanism that is known as the heavy chain switch (Fig. 15-12). The antibody diversity that takes place in the variable region exists by the time the B cell membrane has bound receptor Ig molecules. The cell does not go on to secrete antibodies unless the appropriate antigen binds to the preordained (according to the

clonal selection theory) receptor. Upon antigen binding the cell is driven ("antigen-driven") along its developmental pathway to form a clone of lymphocytes that produce an antibody of a single specificity. B cells begin their antibody-secreting functions by first making IgM. They are able to switch, however, to make the other Ig classes, while retaining the same antigen-binding site. The switch involves a second recombinational event called S/S recombination. Starting from the VJ region the DNA sequences for the constant region of the heavy chains of the Ig classes are lined up in the order of IgM, IgD, IgG, IgE, and IgA. If the heavy chain switch is to IgG, (the most frequent switch), IgM and IgD sequences encoding the constant regions are deleted. If the switch is to IgA, then the sequences encoding the constant regions of IgM, IgD, IgG, and IgE are deleted. The functional implication of the switches is that a particular antigen-binding site can associate with any of the five Ig classes and thereby acquire the biological functions characteristic of each Ig class.

EPILOGUE. Gene rearrangement and recombination as discovered relative to antibody diversity apparently is not unique to this gene system but is a basic genetic mechanism that is operative in other developmental processes.

T LYMPHOCYTES (T CELLS)

Ontogeny, Differentiation, and Maturation

Precursor T cells from the bone marrow migrate as prothymocytes to the thymus gland, where they gradually mature in the late stages of their intrathymic passage into immunocompetent cells (Fig. 15-13). The thymus-conditioned cells are exported as post-thymic cells into the peripheral lymphoid compartments, mainly the lymph nodes and spleen, from whence some enter the circulation (the vascular compartment). The T cells preferentially locate in the corticomedullary junctions of the lymph nodes and appear as cuffs of lymphocytes around arterioles of the spleen.

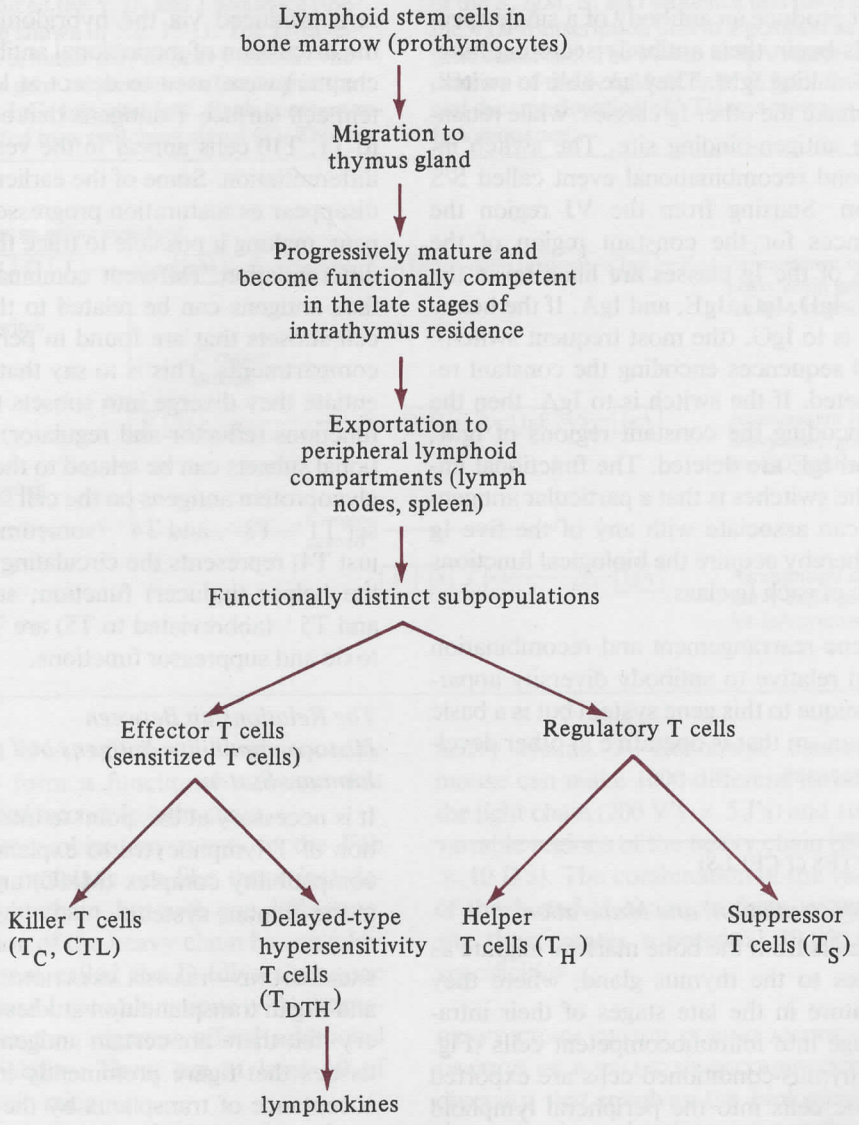
T CELL SURFACE ANTIGENS. T cells exhibit a succession of cell surface glycoprotein antigens as differentiation progresses. The antigens are symbolized by the letter T and a number. Highly specific antisera produced via the hybridoma technique (see the discussion of monoclonal antibodies in the next chapter) were used to detect at least 10 glycoprotein cell surface T antigens that are numbered T10 to T1. T10 cells appear in the very early stages of differentiation. Some of the earlier surface antigens disappear as maturation progresses, and others appear, making it possible to trace the stages of T cell differentiation. Different combinations of the surface antigens can be related to the functions of T cell subsets that are found in peripheral lymphoid compartments. This is to say that as T cells differentiate they diverge into subsets that have distinct functions (effector and regulator), and these functional subsets can be related to the combinations of glycoprotein antigens on the cell surface. Thus subset $T1^+$, $T3^+$, and $T4^+$ (sometimes abbreviated to just T4) represents the circulating T cells that have the helper (inducer) function; subset $T1^+$, $T3^+$, and $T5^+$ (abbreviated to T5) are T cells with cytotoxic and suppressor functions.

The Relationship Between Histocompatibility Antigens and the Immune System

It is necessary at this point to interrupt the description of T lymphocytes to explain the major histocompatibility complex (MHC) and its relationship to the immune system.

BACKGROUND—TRANSPLANTATION ANTIGENS. Tissue and organ transplantation studies led to the discovery that there are certain antigens on transplanted tissues that figure prominently in the rejection or acceptance of transplants by the immune system. The antigens, genetically determined, were found to be glycoproteins that are inserted into the cell membrane. All mammalian species studied thus far have a single chromosome region where the genes that encode the major tissue antigens are located. This region is referred to as the *major histocompatibility complex*. The MHC is more specifically designated as the H-2 system in mice and as the HLA

Fig. 15-13. T cell differentiation.



system in humans. HLA stands for *human leukocyte antigen* (the antigens were first demonstrated on leukocytes), a term internationally agreed on to represent the MHC of humans. The multiple HLA genes are located in close proximity to each other on the sixth chromosome of humans.

The MHC region of the chromosome is further divisible into subregions. The gene loci are characterized by multiple alleles (alternative forms of a gene that code for the same property), and consequently the products of these loci are polymorphic. Different subregions (MHC-A, MHC-B, MHC-C, and MHC-D in humans) code for several classes of glycoproteins. The two principal classes of glycoproteins are designated class I, which is encoded by MHC-A, B, and C, and class II, which is encoded by MHC-D. Class I glycoproteins appear on almost all nucleated somatic cells. Class II glycoproteins, not nearly as widely distributed as the class I, are expressed on most B cells, some T cells, some macrophages, and macrophage-like cells that are involved in presenting antigen to T cells.

The MHC originally was studied for its role in transplant rejection. It now is known also to be involved in immunity to some virus-induced tumors and in the occurrence of many noninfectious diseases. There are associations between genetic traits encoded by the HLA system and predisposition to noninfectious human disease. Associations are particularly strong for ankylosing spondylitis (inflammation of the vertebrae that leads to immobilization and deformity), for susceptibility to Hodgkin's disease, for certain rheumatic diseases, and for a group of diseases that are characterized by chronic inflammation and aberrant immunologic reactions.

MAJOR HISTOCOMPATIBILITY COMPLEX AND THE IMMUNE SYSTEM. Immunologists in the 1970s came to recognize that the MHC, in addition to its roles in transplantation and some infectious diseases, has a profound effect on T cell recognition of antigens and on the effector and regulator functions of T cells.

In the afferent phase of an immune response T cells will only recognize and respond to cellular antigens that are joined with the "self" MHC glycoproteins. Effector T cells will respond only to foreign antigenic determinants expressed in association with MHC antigens on the surface of host or target cells. Cytotoxic T cells from one strain of mice, for example, destroy virus-containing fibroblast cells in tissue culture if the fibroblasts have surface MHC glycoproteins that are identical to those of the cytotoxic T cells. Nonidentical fibroblasts from a different mouse strain and infected with the same strain of virus are not destroyed. The T cells, in other words, have to recognize not only the foreign antigen encoded by the virus but also some self host component, namely an identical cell surface glycoprotein. The advantage of this aspect of the MHC system is that T cells are prevented from being unnecessarily tied up with types of antigens they are unable to dispose of, such as toxins, free viruses, and bacteria. T cell activity would be compromised if the T cells were to bind free viruses because the viruses would occupy T cell receptors and thereby keep the T cell from destroying the virus source, namely the virus-infected cell.

T Cell Antigen Receptors

T cell recognition of antigens involves three molecular components that form a complex: the antigen, a MHC glycoprotein molecule, and one of two T cell receptor molecules. The characterization of the T cell receptor molecules (also called T-cell antigen-recognition molecules or T cell receptors, for short) is not complete. According to some studies, they appear, like Igs, to consist of two disulfide-linked polypeptide chains but lack conventional light and heavy chains. In some cases the same anti-idiotype antibodies (explained later in Regulation of the Immune System) that bind to Igs bind to T cells and inhibit the T cells' ability to respond to antigen. Some support the view, therefore, that since T cell receptors appear to share idiotypes with Ig's, the T cell receptors are encoded in part by the same V_H genes. Others dispute this view and argue that an entirely different set of genes codes for the T cell receptors.

At issue at this time is how many antigen receptors there are in the T cell membrane. The favored

theory, the single receptor theory, suggests that the MHC surface glycoprotein molecules first interact with the antigen and form a new antigen. This new entity then binds to a single T cell receptor. The second theory, a two-receptor model, suggests that one T cell receptor binds the antigen and the other binds the MHC glycoprotein molecule.

Differences Between T Cells and B Cells

There are functional, antigenic, developmental, and morphological differences between B cells and T cells. T cells do not differentiate into plasma cells on antigen contact. Instead, they enlarge into medium-to-large lymphocytes referred to as *sensitized lymphocytes*. B cells have membrane-bound Igs that serve as antigen receptors, whereas T cell antigen receptors have not been completely identified. T cells have a life span of months; the life span of B cells is numbered in days to weeks. T cells can be stimulated to undergo mitosis by the plant substances phytohemagglutinin and concanavalin A. Sheep red blood cells adhere to T cells in vitro and form what are known as E (erythrocyte) *rosettes*. T cells bear the greater responsibility for immunoregulation, as will be seen later in this chapter.

Functional Roles of T Cells—Effectors and Immunoregulators

Functionally, T cells are divisible into subpopulations of effector cells and regulator cells (Fig. 15-13). The effector role is directed at foreign antigens expressed on host target cells; the regulator role, at B, other T, and macrophage cells.

T EFFECTOR CELLS. Effector cells are those that have been stimulated by an antigen in the afferent arc of the immune response and then as antigen-sensitized cells go on to provide cell-mediated immunity (CMI) or to account for delayed-type hypersensitivity in the efferent arc. One way to refer to effector T cells that have been activated by an antigen is as *sensitized lymphocytes*.

The protective functions of T lymphocytes include immunity to certain infectious agents, especially those that have an intracellular habitat or those that tend to be involved in chronic infections (for example, tuberculosis and histoplasmosis). Cancer surveillance is believed to be an important function of CMI: As cells multiply in the body, it is not unusual for some cells to become altered and multiply uncontrollably. The CMI "recognizes" such cells with their tumor antigens as non–normal self antigens and destroys the cells. The rejection of foreign grafts is also largely the function of a CMI response.

The T cells of CMI also produce other harmful effects. They are the cells that control the mediators of delayed hypersensitivity. Delayed hypersensitivity diseases include infectious hypersensitivity, allergic contact dermatitis, aspects of autoimmune disease, and other clinical entities. Delayed hypersensitivity is covered in Chapter 17. CMI and humoral immunity mechanisms do interact, and both may respond to the same antigen.

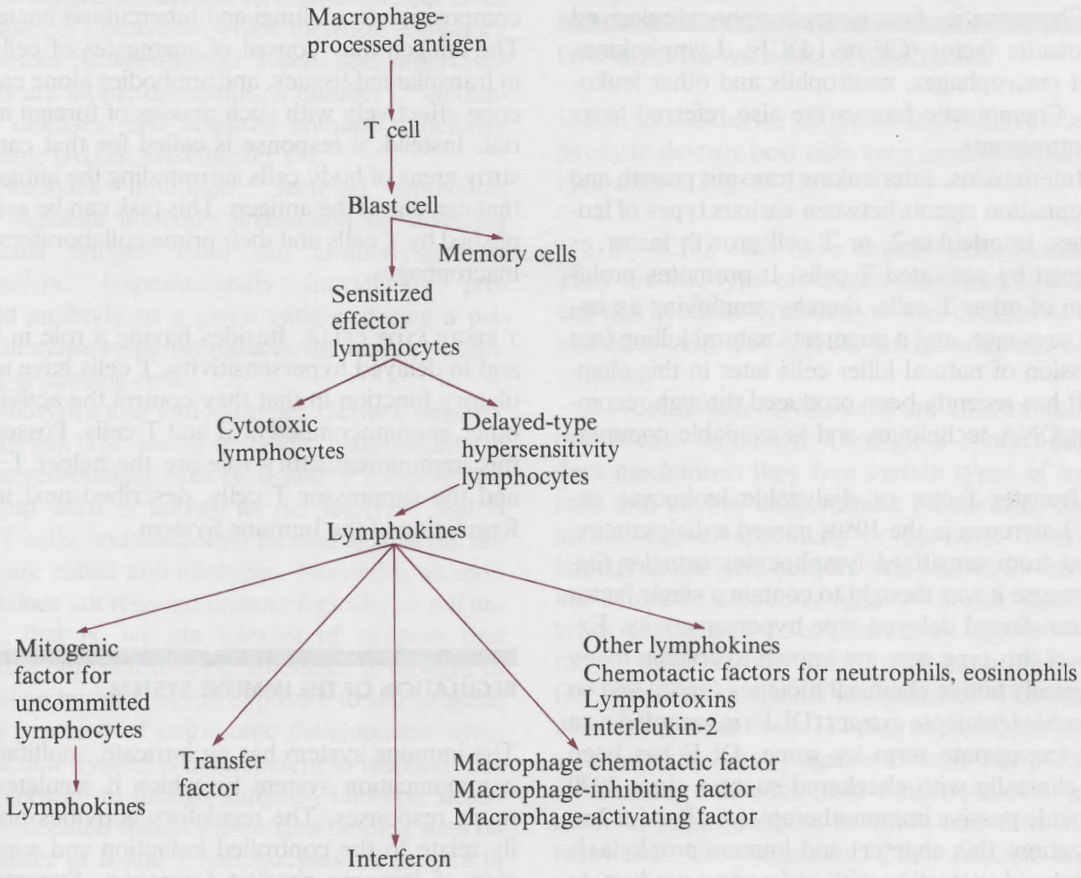
There are two subsets of effector T cells, cytotoxic T cells (T_C) and T cells involved in delayed-type hypersensitivity (T_{DTH}) (Fig. 15-14).

Cytotoxic T Cells. T_C cells are also referred to as T_K or killer T cells and as cytotoxic T lymphocytes (CTL). Cytotoxic T cells directly destroy cellular antigens. When cytotoxic T cells are exposed to foreign cells (e.g., transplanted cells) or virus-infected cells that have viral antigens on their surfaces, the T_C effector cells bind to and kill the target cells. The molecular events responsible for the killing are not known, but contact between the T_C cell and the target cell is required. A single T_C cell can kill many target cells.

Delayed-Type Hypersensitivity T Cells. The number of committed T cells often is too low to cope alone with the antigens. T_{DTH} cells do not directly destroy the antigen themselves. Their activities are greatly expanded through their ability to recruit macrophages, neutrophils, and other cells to the site of antigen confrontation. They do this by secreting soluble, short-range messenger substances called *lymphokines* that invoke the assistance of other cell types, especially macrophages, and it is cells of this other type that finally destroy or dispose of the antigen. B cells also produce some lymphokines, according to recent evidence.

Lymphokines. Lymphokines are named accord-

Fig. 15-14. Responses of an immunogen-exposed T cell. T cells with a specific receptor (the nature of the receptor is not known) for a given antigenic determinant transform into blast cells upon antigen exposure. The lymphoblast cells transform into memory cells and into sensitized lymphocytes. Sensitized lymphocytes may directly deal with the antigen (cytotoxic lymphocytes) or release lymphokines. Lymphokines have not been chemically characterized. They are named according to their biological activity.



ing to their biological activity. Their chemical structures are largely unknown because they are produced in extremely small (nanogram) quantities, purification is extremely difficult, and homogeneity is difficult to attain. It may well be that among the many named lymphokines some will be found to be chemically identical. Lymphokines, unlike secreted products of the B cells, do not have antigen specificity. Lymphokines affect the proliferation, activation, and chemotaxis of other cells such as uncommitted lymphocytes, macrophages, granulocytes, mast cells, and osteoclasts (resorb bone) (see Fig. 15-14). Among the better known lymphokines are the following:

1. Macrophage activating factor (MAF). Macrophages activated by this lymphokine have increased mobility and increased capacities for phagocytosis and killing.

2. Macrophage migration inhibiting factor

(MIF). It can be shown that macrophages in a capillary tube are inhibited from migrating out of the tube in the presence of sensitized lymphocytes and their homologous antigen. Whether this effect occurs in vivo is unclear, and there is evidence that MIF is merely one manifestation of MAF.

3. Chemotactic factor or lymphocyte-derived chemotactic factor (CF or LDCF). Lymphokines attract macrophages, neutrophils and other leukocytes. Chemotactic factors are also referred to as leukoattractants.

4. Interleukins. Interleukins transmit growth and differentiation signals between various types of leukocytes. Interleukin-2, or T cell growth factor, is produced by activated T cells. It promotes proliferation of other T cells, thereby amplifying an immune response, and it augments natural killing (see discussion of natural killer cells later in this chapter). It has recently been produced through recombinant DNA techniques and is available commercially.

5. Transfer factor or dialyzable leukocyte extract. Lawrence in the 1950s named a dialysate extracted from sensitized lymphocytes *transfer factor* because it was thought to contain a single factor that transferred delayed-type hypersensitivity. Extracts of this type now are known to contain many biologically active chemical moieties (parts) and so *dialyzable leukocyte extract* (DLE) is regarded as a more appropriate term by some. DLE has been used clinically with checkered success since 1970 to provide passive immunotherapy (see Passive Immunization, this chapter) and immunoprophylaxis (beforehand protection with an immune product) to patients whose CMI is defective (see Fig. 17-8).

6. Other lymphokines. Many other lymphokines have been reported: leukocyte inhibitory factor, osteoclast activating factor, lymphocyte transforming factor, specific macrophage arming factor, skin reactive factor, lymph node permeability factor, and interferon (especially important as a nonspecific inhibitor of virus replication). Some of the reported factors have not been well characterized.

The nature of the antigen largely determines whether the CMI system or the humoral immune system will eliminate it. Antigens such as viruses and other microorganisms in an intracellular habitat are not directly exposed to antibodies. Antibodies do not act intracellularly. The response calls for a mechanism that can destroy the housing cell. The antigen may be difficult to destroy because of its composition (e.g., fungi and tuberculosis bacteria). The antigen may consist of aggregates of cells, as in transplanted tissues, and antibodies alone cannot cope effectively with such masses of foreign material. Instead, a response is called for that can destroy areas of body cells surrounding the antigen or that can digest the antigen. This task can be accomplished by T cells and their prime collaborators, the macrophages.

T REGULATOR CELLS. Besides having a role in CMI and in delayed hypersensitivity, T cells have a regulatory function in that they control the activity of other immunocompetent B and T cells. Possessing this immunoregulatory role are the helper T cells and the suppressor T cells, described next under Regulation of the Immune System.

REGULATION OF THE IMMUNE SYSTEM

The immune system has an intricate, multifaceted communication system by which it regulates immune responses. The regulatory activities primarily relate to the controlled induction and suppression of immune product formation. Suppression especially is necessary to curb a continuing response once the antigen has been eliminated. The best known elements of immunoregulation are as follows:

1. Helper or inducer T cells (T_H). Helper T cells are essential for B cells to respond to most antigens. (Some antigens, especially large polymers that have repeating identical antigenic determinants, can activate B cells without the assistance of T cells). Helper T cells may also assist effector T cells and suppressor T cells to respond to antigen.

2. Suppressor T cells (T_S). Suppressor T cells can reduce or down regulate the response of B cells or other T cells to antigens. T_S cells interact with T_H cells in a self-regulating feedback circuit. Helper cells constantly incite suppressor cells into action, and the suppressor cells in turn inhibit helper cells. Examples of diseases in which there are regulatory imbalances between helper T cells and suppressor T cells are atopic dermatitis, a number of autoimmune diseases, and acquired immune deficiency syndrome (AIDS, see Chapter 17).

3. Feedback inhibition. Secreted antibodies partly regulate antibody production by diverting additional antigen from the antibody-forming mechanism. Experimentally introducing preformed antibody to a given antigen during a primary immune response reduces the number of antibody-producing cells.

4. Idiotypes and anti-idiotypes (Jerne's network hypothesis). The set of antigenic determinants of the antigen-binding sites of B and T lymphocytes make up what is known as the idiotype, and B cells, T cells, and antibodies formed against the idiotype are called anti-idiotypes. Normally, an individual does not respond immunologically to self antigens, that is, we are tolerant of antigens that comprise our own tissues. Tolerance is thought to depend on immunological exposure to self antigens during a period of embryonic development when the immune system is incompetent to respond. Before exposure to foreign antigens, idiotypic determinants located within the antigen-binding sites on the surface of B and T lymphocytes are present in concentrations too low to establish tolerance. When the appropriate antigen stimulates the T and B cells, there is a marked increase in idiotypic determinants (i.e., antibodies and sensitized lymphocytes bearing the idiotypic determinants) that are not recognized as self. This triggers the synthesis of anti-idiotypes. The anti-idiotype in turn expresses antigenic determinants that can stimulate the synthesis of anti–anti-idiotypes. In this way a network is formed that reverberates throughout the immune system. An inference from the network of interactions is that a dynamic equilibrium is attained. Anti-idiotypes exert a positive or negative effect on immune product formation by controlling the suppression or expansion of clones of lymphoid cells.

LYMPHOCYTES WITH KILLER FUNCTIONS

Different subclasses of lymphocytes have the capacity to destroy host cells via a contact-dependent mechanism (Fig. 15-15):

1. *T_C (CTL) cells* have already been described. They are one type of antigen-sensitized effector T cell that specifically destroys virus-containing cells and other cells that express foreign antigenic determinants.

2. *Natural killer (NK) cells* are effector cells of nonspecific immunity. Through a contact-dependent mechanism they lyse certain types of tumor cells and normal cells without manifesting classic immunological specificity or memory. They are large granular lymphocytes best known for mediating cytotoxic reactions against various histologic types of maligant cells. Since they appear "naturally," they provide (along with macrophages) a first-line defense against malignancies. This role of destroying cancer cells is being explored for its use as a possible therapy against cancer. The proposal is to obtain NK cells from cancer patients, grow them in large numbers in vitro, and then infuse them back into the patient. Other possible immunopathologic effector roles that are being ascribed to NK cells include rejection of bone marrow transplants, regulation of cellular development, and protection against infection.

3. *K cells* are responsible for antibody-dependent cell-mediated cytotoxicity. K cells have a surface receptor that binds to the Fc portion of IgG molecules. Thus, when a host cell is coated with IgG (the Fab portion is attached to the host cell), the cell becomes a target for killing by the K cell. It is generally believed that K cells are closely related or identical to NK cells.

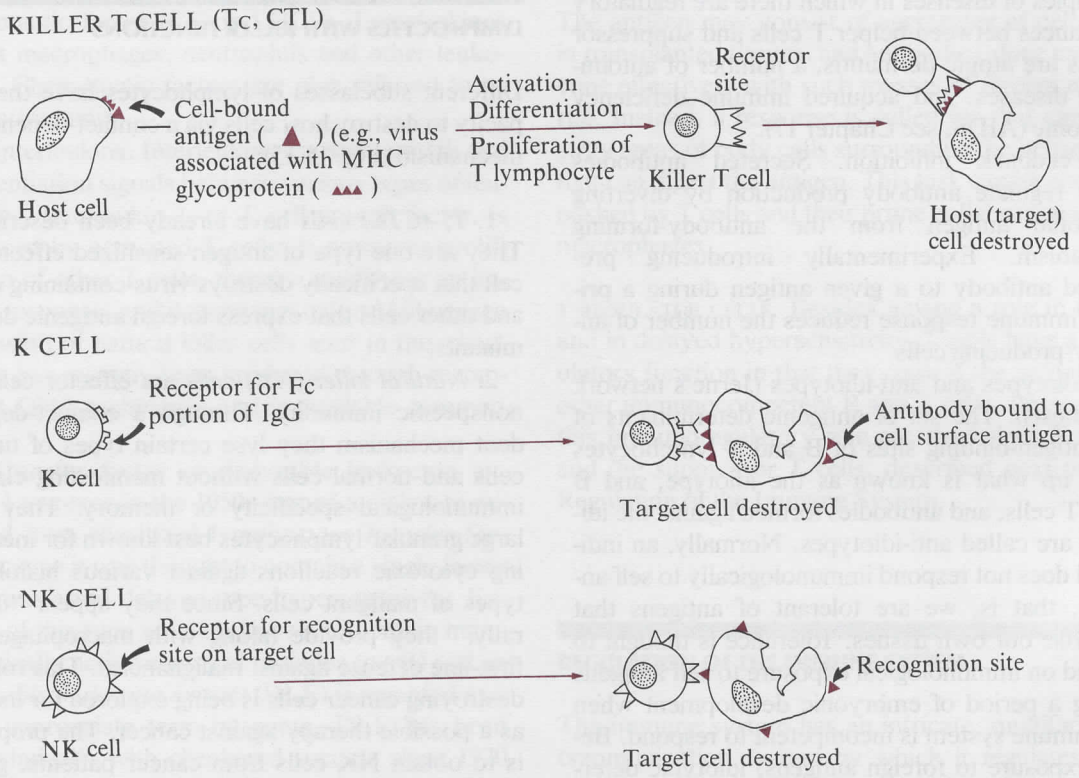


Fig. 15-15. Top, Killer T cells. Before they become sensitized, killer T cells must be exposed to the antigens of the target cell surface. The target cell must bear the class I glycoprotein of the major histocompatibillity complex (MHC). Middle, K cells. When a tissue cell has cell surface antigens to which IgG antibodies are bound via the antigen-binding sites, the cell becomes a target for destruction by K cells. K cells have receptors for the Fc portion of the IgG molecule. Bottom, Natural killer (NK) cells. NK cytotoxic activity does not require antibodies. The receptors on the NK cells and the recognition site on the target cell have not been characterized.

THE COMPLEMENT SYSTEM

NATURE OF THE COMPLEMENT SYSTEM

There are several vital systems in the blood of vertebrates that operate in a sequential fashion once their inactive precursors (zymogens) are triggered to go into action. They are the blood-clotting and clot-lysing systems, the kinin system (kinins are small peptides that induce contraction of smooth muscle, increase vascular permeability, and influence the mobility of cells), the renin-angiotensin system (renin produced by the kidney activates the angiotensin system, thereby mediating prompt changes in blood pressure), and the complement system.

The complement system (Fig. 15-16) has two synergistic pathways, the classic pathway and the alternative pathway (also called the alternate pathway or the properdin pathway). There is a basic series of 11 serum proteins numbered C1 through C9. Complement factor C1 is made up of three subclasses—C1q, C1r, C1s—thereby accounting for

the 11 serum proteins. There also are 7 proteins that are involved in the activation and regulation of the alternative pathway. These proteins in the inactive form comprise about 10 percent of the globulin portion of normal serum. Once it is activated the complement system proceeds in a serial, cascade manner, each reaction setting up another reaction. Many of the proteins are converted from proenzymes to enzymes. Some fragments adhere to other proteins or to surfaces. During the cascade a number of biologically active molecules or complexes are generated that facilitate the localization or destruction of foreign substances. The activities of the system are controlled by inhibitors that are specific for certain of the components and by the instability of some of the enzyme intermediates.

CLASSIC COMPLEMENT PATHWAY

The classic pathway is activated when IgM or IgG antibody reacts with an antigen. The Fc portion of the antibody forms a binding site for C1q. C1q has six binding areas, at least two of which must bind to the appropriate domains of neighboring constant regions of Ig molecules for activation to occur. The other immunoglobulin classes cannot activate the classic pathway because they do not have a binding site for C1q. One outcome of the classic pathway is lysis of the cell to which the antibody is attached. Other biological activities also occur, as is explained later.

The numerical sequence of participation of the complement factors in the classic pathway is C1, C4, C2, C3, C5, C6, C7, C8, C9. The alternative pathway starts at C3. The reaction sequence of the entire classic pathway can be divided into three stages: recognition unit, activation unit, and membrane attack unit. These stages occur at separate topological sites on the cell (antigen) surface. At site I, C1q serves as the recognition element because it binds to IgM or IgG at that site. The activated C1 molecule can cleave many molecules of C4, thus giving rise to a greatly expanded action of the complement system. The expanded role is sometimes termed *biological amplification*. After recognition, the next factors to participate are C4, C2, and C3, at site II. These three factors constitute the activation unit, which involves important enzyme activities. The remaining C factors, C5, C6, C7, C8, and C9, comprise the membrane attack complex at site III, where cell membrane damage occurs. The antigen need not be a part of the cell for lysis of the cell to take place. The antigen may merely be attached or adsorbed to the cell. What has just been described involves those complement components of the classic pathway that bind to the cell membrane surface. Also formed during the sequence are complement factor split products (or fragments) such as C3a, C3b, and C5a. They appear in the serum (fluid phase), where they may have anaphylatoxic, chemotactic, opsonic, and other activities, which will be explained later.

ALTERNATIVE OR PROPERDIN PATHWAY

The alternative pathway enters the complement sequence at the C3 juncture. Since C1, C4, and C2 are bypassed, activation of the C1 recognition unit is bypassed, as is the assembly of the C4, C2, C3 activation unit of the classic pathway. Antibodies are not required to initiate activation of this sequence, so entrance at this level of the complement sequence provides a means of amplifying nonspecific resistance (see Biological Activities of Complement).

Many complex polysaccharides will initiate the activation of the alternative pathway: endotoxins, dextran, polysaccharide capsules, and aggregated IgE and IgA macromolecules. At present, at least six serum proteins are recognized as functioning in the activation of the alternative pathway in response to the presence of the complex polysaccharides. One of the proteins, initiating factor, acts as the recognition element in a manner analogous to C1q's role in the classical pathway. The net result of this activating complex of proteins is the generation of an enzyme (C3 convertase), which cleaves the pivotal complement factor C3 into C3a and C3b. C3b is deposited on the surface of the particle that triggered the activation. When a critical

density of C3b is attained, C5 convertase develops. C5 convertase cleaves C5 into C5a and C5b. The active fragments generated in these steps mediate various biological activities as the situation requires.

The role of properdin in the alternative or properdin pathway has been clarified recently. For years properdin was regarded as a series of serum proteins that directly activated the alternative pathway. It is now known to be the final protein of the six proteins that comprise the alternative pathway assembly. Properdin stabilizes the C3b and factor B complex (C3 proactivator), protecting the complex from rapid inactivation by C3b inactivator.

BIOLOGICAL ACTIVITIES OF COMPLEMENT

Cytolysis

The membrane attack complex, which consists of C5b, C6, C7, C8, and C9, creates a tubular hydrophobic structure in the lipid bilayer of the cell membrane. Osmotically active materials pass through the lesion. Solutes and water enter, the cell swells, and osmotic lysis finally takes place. When antibodies are involved, the phenomenon is called *immune cytolysis*—more specifically, *immune hemolysis* when red blood cells are involved and *immune bacteriolysis* when bacterial cells are involved. It is also possible for cytolysis to occur via the alternative pathway even though the initiators of this pathway are not intact cells. The complex that is formed attaches to cells such as red blood cells and platelets, and lysis occurs (hematological cytolytic disease). White blood cells probably do not undergo cytolysis of this type because they reportedly have the protein synthetic machinery to repair the initial lesion before biological amplification occurs.

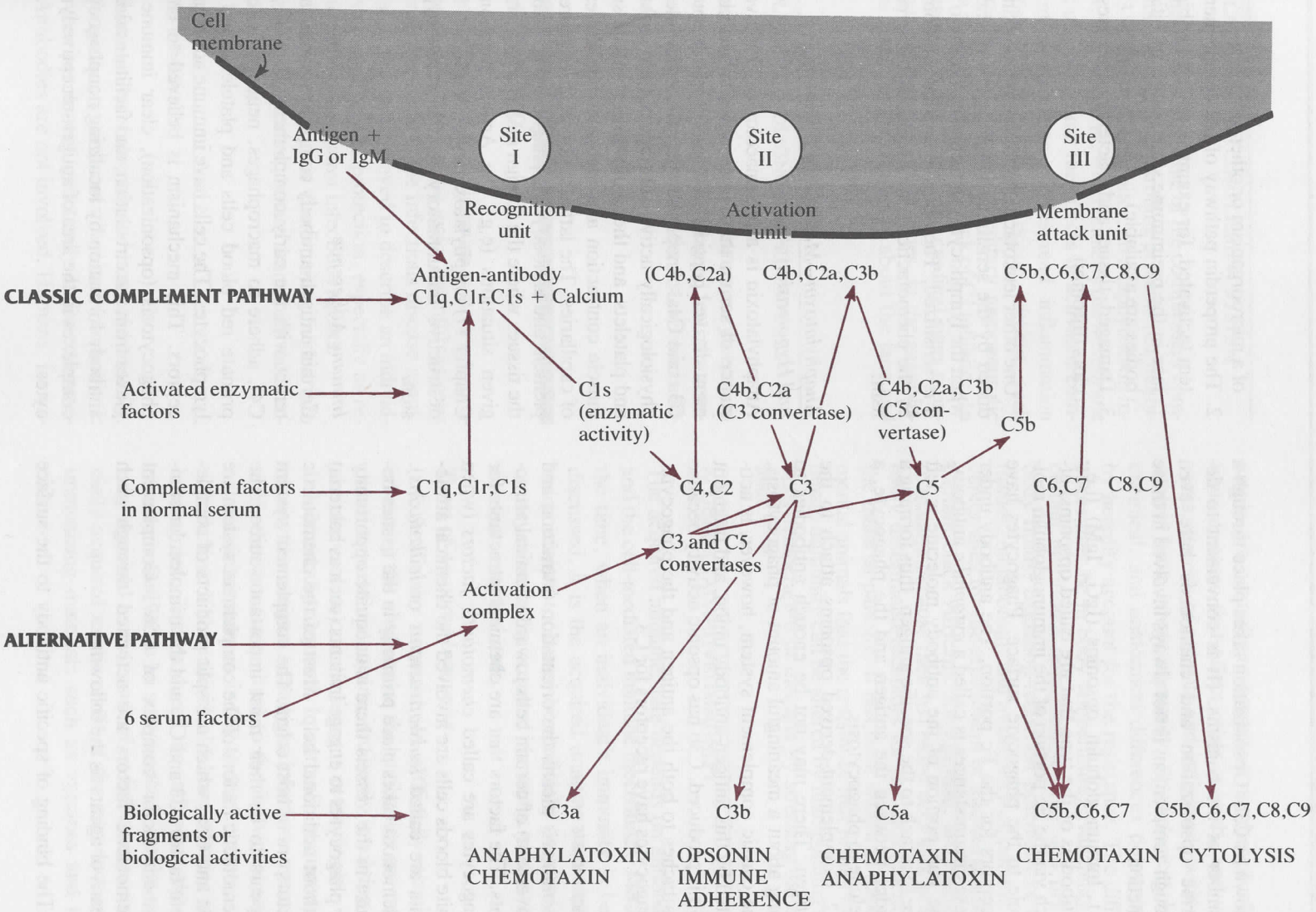
Opsonization

The virulence of some microorganisms is due in part to their ability to escape phagocytosis. The body has means to capture and destroy these evaders through serum proteins, called *opsonins*, that include the split product of complement factor C3

Fig. 15-16. The complement system. In the classic pathway the nine complement factors act in a sequence with the end result of immune cytolysis. Antigen and IgG or IgM bind C1q. The recognition by C1q leads to the formation of the activated recognition unit at site I on the cell membrane. An enzyme, C1s, formed by the recognition unit converts serum factors C4 and C2 to an enzyme, C4b, C2a, which converts the pivotal C3 into the full activation unit at site II. The activation unit (C4b, C2a, C3b) activates C5 of the serum to form C5b and C5a. C5b, with C6, C7, C8, and C9, forms the membrane attack unit at site III.

In the alternative (or properdin) pathway six serum proteins form an activation and stabilization complex that enters the complement sequence at the pivotal C3 level. Most of the biological activities of complement can be activated by this pathway. Antibodies are not required for its activation, which provides a mechanism of engaging complement biological activities in the nonimmunized state. The enzymes that activate C3 and C5 are different from the convertases of the classic pathway.

Biological activities are generated by both pathways during the cascade.


Cell membrane
Antigen + IgG or IgM
Site I
Site II
Site III
Recognition unit
Activation unit
Membrane attack unit
CLASSIC COMPLEMENT PATHWAY
Antigen-antibody C1q,C1r,C1s + Calcium
(C4b,C2a)
C4b,C2a,C3b
C5b,C6,C7,C8,C9
Activated enzymatic factors
C1s (enzymatic activity)
C4b,C2a (C3 convertase)
C4b,C2a,C3b (C5 convertase)
C5b
Complement factors in normal serum
C1q,C1r,C1s
C4,C2
C3
C5
C6,C7
C8,C9
C3 and C5 convertases
Activation complex
ALTERNATIVE PATHWAY
6 serum factors
Biologically active fragments or biological activities
C3a
C3b
C5a
C5b,C6,C7
C5b,C6,C7,C8,C9
ANAPHYLATOXIN CHEMOTAXIN
OPSONIN IMMUNE ADHERENCE
CHEMOTAXIN ANAPHYLATOXIN
CHEMOTAXIN
CYTOLYSIS

known as C3b. Opsonization takes place through a number of mechanisms. (It is convenient to describe opsonization and chemotaxis here even though complement is not always involved in these functions.)

1. Immunoglobulin opsonins (IgG, IgM) (i.e., antibodies of the type that are called opsonins) attach via the Fc portion of the immunoglobulin molecule to the phagocyte surface. Phagocytes have receptors for the Fc portion. The antibody under these circumstances is called a *cytophilic* antibody. The Fab portion of the antibody molecule is left free to attach to the specific antigen, thus forming a bridge between the antigen and the phagocyte, a prelude to phagocytosis.

2. Complement-derived opsonins attach to the antigen. There may not be enough antibodies to bring about a meaningful amount of phagocytosis. The classic complement system, however, is activated by this antigen-antibody union, and fragment C3b is produced. C3b has opsonic activity because it attaches to both the antigen and the phagocyte. Phagocytes have receptors for C3.

Chemotaxis

Chemotaxis means the orientation (attraction and movement) of certain cells toward chemical attractants. The factors that are chemical attractants for phagocytes are called *chemotactic factors* (when white bloods cells are involved the chemical attractants are called *leukoattractants* or *leukotaxins*). Chemotaxis takes place primarily in the tissues because in the vessels there is adequate opportunity for phagocytes to engage irritants (such as bacteria) without additional help. Most of the chemotactic factors are of host origin. The complement system appears to be their most important source. The chemotactic factors of the complement system are C3a and C5a, which are split products of complement factors C3 and C5, and the trimolecular complement factor complex of C567. Complement chemotactic factors are activated through such means or agents as the following:

1. The binding of specific antibody to the surface of a microorganism or other antigen
2. The properdin pathway of the complement system (activated, for example, by a microbial surface in the preimmune state before specific antibodies are available)
3. Damaged tissue and bacterial proteases, and clots (thrombi)

One other leukotactic factor is a lymphokine produced by the sensitized lymphocytes of the CMI when the lymphocyte contacts the antigen to which it is sensitized. The phagocytes that are contacted by the chemotactic factors increase their metabolism.

Anaphylatoxin—Mediator of Inflammation and Hypotension

Anaphylatoxin is a pharmacologically active substance of serum that is composed of the complement-derived peptides C5a and (of less importance) C3a and C4a. Anaphylatoxin causes the release of physiologically active substances from mast cells and platelets, and these substances initiate smooth muscle contraction and increase the permeability of capillaries. The latter action enables more complement and antibody and phagocytic cells to enter the tissues where the foreign material is located. In given situations (e.g., the Arthus reaction—see Chapter 17) anaphylatoxin induces intense, tissue-destructive inflammatory reactions and hypotension.

Immune Adherence

Certain antigen-antibody complexes, when they interact with the early complement proteins (C1 to C4), adhere to macrophages, neutrophils, certain primate red blood cells and platelets, and some lymphocytes. The cells have immune adherence receptors. This mechanism is believed to enhance phagocytosis (opsonization), clear immune complexes from the circulation, and facilitate additional antibody formation by localizing nonphagocytosed complexes in the area of antigen-receptive lymphocytes.

REGULATION OF THE COMPLEMENT SYSTEM

The short half-life of some of the complement components and certain controlling proteins (C1 inhibitor, C3 inhibitor, C4 inhibitor, an anaphylatoxin inhibitor, and S protein, which inhibits the membrane attack complex) limit the effects of activated complement components. Widespread inflammation and tissue damage could occur without such constraints. The disease hereditary angioneurotic edema is due to the patient's lack of C1 inhibitor. In this disease edema, one of the cardinal signs of inflammation, occurs in extremities, in the gastrointestinal tract, and occasionally about the face and neck.

COMPLEMENT IN DIAGNOSTIC PROCEDURES

Complement involvement in immune cytolysis is the basis of a well-known serological test, the complement fixation (CF) or comp fix test, which is described in Chapter 16.

TYPES OF IMMUNITY

The prime function of the immune system is to provide protection. One of the major harmful antigens that enter the body is the infectious disease agent. Special terms are employed to describe an individual's state and type of protection, especially as related to protection against infectious agents.

The immune status is customarily divided into two major categories, innate immunity and acquired immunity.

INNATE IMMUNITY

Innate immunity is also termed *natural immunity, genetic immunity*, and *constitutional immunity*. It refers to a permanent, genetically endowed immunity. Antibodies are not involved. Humans, for example, are naturally resistant to a number of infectious diseases that affect other animals, and vice versa. Breeds of animals and individuals within a species have innate immunity. Physiological, biochemical, and anatomical differences determined by heredity account for the resistance. The differences may be in body temperature, availability of certain nutritional requisites, tonicity and pH of body fluids, oxidation-reduction potentials of the tissues, metabolism, and availability of suitable receptor sites for the attachment of microorganisms. Certain black Africans, for example, are resistant to malaria because their red blood cells lack genetically determined receptors for the attachment of the malarial parasite. It has been shown in experimental animals that the resistance that results from innate immunity can be altered by manipulating the body temperature or diet and by stressing the animals.

ACQUIRED (ADAPTIVE) IMMUNITY

The acquired immune state depends on antibodies and the cell-mediated immune mechanism. Most of the time, when an individual's immunity is being discussed, it is the acquired immune state that is being referred to. It may be artificially acquired immunity or naturally acquired immunity, an active or a passive immune state.

Types of Acquired Immunity

1. Artificially acquired. In artificially acquired immunity the antigens or the antibodies are introduced by artificial means, ordinarily involving the use of a vaccine or an antiserum, respectively.

2. Naturally acquired. In naturally acquired immunity the antigen, usually an infectious agent, or antibodies are transmitted to the individual under natural circumstances, in day-to-day contacts.

3. Active. If the individual whose immune state is being described produced the antibodies, the immunity is said to be an active one. It can be naturally acquired as the result of an infection or artificially acquired as the result of the introduction of immunizing materials such as vaccines and toxoids.

4. Passive. If the individual whose immune state is being described did not produce the antibodies, the immunity is said to be a passive one. The antibodies may have been injected in the form of an antiserum or immune globulins that were obtained from animals or from other humans (artificially acquired passive immunity). The antibodies may have been transmitted under natural circumstances, as in the transfer of maternal antibodies across the placenta to the fetus or the transmission of antibodies via colostrum (naturally acquired passive immunity). The passive immune state is of short duration—weeks to months. The proteins of administered serum are foreign, and the body reacts to them as to foreign antigens.

COLLATION. The qualifying terms applied to the acquired immune state have been described. It is customary to use three terms in citing the immune state: Is it acquired or is it innate? If it is acquired, is it naturally acquired or artificially acquired? Is it active or passive? Four examples demonstrate how the qualifying terms are used:

1. Naturally acquired active immunity results after recovery from an infection.
2. Naturally acquired passive immunity is an infant's transitory immune state as a result of maternal antibodies that were transmitted transplacentally.
3. Artificially acquired active immunity results from active immunization (vaccines, toxoids).
4. Artificially acquired passive immunity is the transitory immune state that results from the injection of an antiserum.

The terms are not necessarily always used in the order given in the examples. For example, a naturally acquired active immunity can just as well be called an active immunity, naturally acquired.

IMMUNIZATION PRACTICES

It is possible and practical to protect (immunize) an individual specifically against certain antigens, most especially against certain infectious agents or their products (exotoxins).

ACTIVE IMMUNIZATION

The material that is introduced to evoke the active immune state is referred to as *immunizing material*, *immunogen*, or *antigen*. More specifically, it may be designated *toxoid* when it is a modified toxin or *bacterin* when it is a suspension of modified bacteria. The term *vaccine* has various definitions. In the historical sense a vaccine is the material obtained from cattle with an artificial infection of cowpox that was or is employed to immunize against smallpox. A vaccine is generally thought of as consisting of living or killed microorganisms that are used as immunogens. Some people include under the category of vaccine microbial products that are converted into immunizing materials (e.g., toxoids).

Antigens that are used for active immunization are processed in the laboratory so as to alter their disease-causing capability. Tetanus and diphtheria exotoxins are converted to toxoids by the addition of formalin. The conversion can be accomplished in several other ways: ultraviolet irradiation, mild heating, and aging. Some preparations consist of suspensions of killed microorganisms—for example, the whooping cough (pertussis) vaccine. A number of effective immunogens consist of living microorganisms that, through laboratory processing and selection, have lost their virulence or have had their virulence greatly diminished. It is a general tenet of active immunization practices that living microorganisms induce a higher level of immunity than do nonliving ones. Examples of immunogens that contain live attenuated microorganisms are the measles, mumps, and oral polio vaccines and the BCG vaccine that is used for active immunization against tuberculosis.

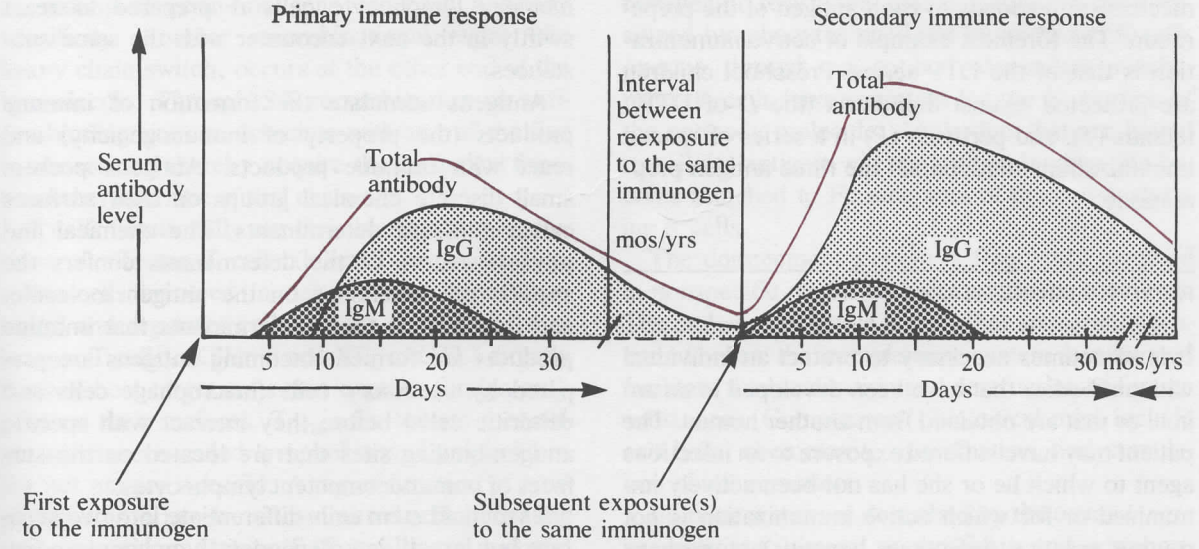


Fig. 15-17. Primary and secondary immune responses. In the primary response IgM is the first to appear in the serum. It appears on the fifth to seventh day and peaks at about 14 days. IgG appears about the tenth day and peaks several weeks later. In the secondary immune response, both IgM and IgG appear to increase within 2 to 3 days. In the secondary immune response, the lag period is shorter, the total antibody level far surpasses that of the primary immune response, antibody is formed over a longer period of time, and much more IgG is formed than IgM.

Prolongation and Intensification of the Immune Response

The antibody level attained and its persistence are enhanced by the addition of adjuvants to the antigen preparation. Adjuvants are believed to prolong the antigenic stimulus, thereby elevating the antibody response. Alum-precipitated toxoids, for example, produce higher antibody titers and longer-lasting immunity than do toxoids alone. Freund's complete adjuvant, consisting of mycobacteria in a water-in-oil emulsion, is used experimentally by immunologists especially to heighten the CMI response.

Dynamics of the Immune Response

PRIMARY IMMUNE RESPONSE. The first exposure to the antigen, whether through infection or vaccination, leads to the primary immune response (Fig. 15-17). A readily detectable level of antibodies does not develop immediately. There is a lag period or induction period of days before detectable protective levels of antibody are formed. Artificial active immunization would not be needed if a protective level were reached in hours. With a single antigen exposure the antibody titer frequently drops after a comparatively short time, sometimes to a level that is no longer protective.

SECONDARY IMMUNE RESPONSE. With a number of immunogens it is the practice to give additional exposures to the antigen at appropriately spaced intervals. These ensuing stimuli are commonly termed *booster* doses. The additional antigenic stimulus elicits prompt antibody formation, and the antibody level is higher and persists longer. The prompt, elevated response to additional antigenic stimuli is due to the anamnestic response, also termed the *recall phenomenon*. Antibody-forming cells "turned on" by the first exposure to the anti-

gen "remember" how to form the antibodies specific for that antigen and do so promptly and efficiently. The number and spacing of booster doses differ according to the requirements of different age groups and populations, and for the various disease agents. An antigen may be given in combination with other antigens because the antibody-forming mechanism responds to each antigen of the preparation. The foremost example of active immunization is that of the DTP series. Preschool children are protected against diphtheria (the *D* of DTP), tetanus (*T*), and pertussis (*P*) in a series of spaced immunizations that contain the three antigen preparations.

PASSIVE IMMUNIZATION

It is sometimes necessary to protect an individual with antibodies that have been developed in an animal or that are obtained from another human. The patient may have suffered exposure to an infectious agent to which he or she has not been actively immunized or for which active immunization is not routine practice (infectious hepatitis) or perhaps against which is is not feasible to immunize actively (botulism). In such circumstances, even if active immunizing material were available, it is not possible for the patient to produce a sufficient level of his or her own antibodies to fight off the current infection. Antisera developed in animals or gamma globulins obtained from humans are used to provide temporary, immediate protection. The antisera contain a high level of specific antibodies, while some gamma globulin preparations contain the gamma globulin fraction of pooled plasma. When gamma globulin from a number of individuals is pooled, it is likely to contain antibodies to the common infectious diseases. Passive immunization is also referred to as *passive immunotherapy* or, when serum is used, as *serotherapy*.

SUMMARY

The immune system specifically recognizes foreign substances with certain characteristics (antigens), forms immune products (antibodies and sensitized lymphocytes) against the substances, and through memory lymphocyte cells is prepared to react swiftly in the next encounter with the same substances.

Antigens stimulate the formation of immune products (the property of immunogenicity) and react with immune products. Antigens possess small discrete chemical groups on their surfaces called antigenic determinants. The chemical and physical nature of the determinants confers the specific characteristics on the antigen molecule, and it is against these determinants that immune products are formed. Incoming antigens are prepared by accessory cells (macrophage cells and dendritic cells) before they interact with specific antigen-binding sites that are located on the surfaces of immunocompetent lymphocytes.

Lymphoid stem cells differentiate into two separate but largely interdependent lymphocyte populations: the B lymphocyte (B cell) population, which is responsible for the production of antibodies that give rise to humoral immunity; and the T lymphocyte (T cell) population, which is responsible for the production of sensitized lymphocytes that give rise to cell-mediated immunity (CMI).

B cells produce 5 classes of immunoglobulins, namely IgM, IgD, IgG, IgE, and IgA. The classes differ in amino acid composition and sequences, in their biological activities, and antigenically (isotypes). A basic Ig molecule is composed of two heavy (H) chains and two light (L) chains that are joined together by disulfide bonds. The Fab portion of the molecule contains variable (V) and constant (C) regions of H and L chains. The V regions form the antigen-binding site of an antibody molecule. The other end of the Ig molecule, the Fc portion, is composed of C regions of H chains. Biological functions of the Fc include binding of Ig's to host cells (e.g., mast cells, macrophages, neutrophils),

binding of complement by IgG and IgM, and facilitating of the transplacental passage of IgG.

Antibody diversity is due to intrachromosomal recombination in somatic cells of selected V, J, and C gene sequences to form a single transcription unit. This mechanism allows the generation of a large repertoire of antibody genes without requiring an individual germ-line gene for each antibody specificity. Another recombinational event, the heavy chain switch, occurs at the other end of the Ig molecule. Through S/S recombination an antigen-binding site can associate with any of the five Ig classes and thereby acquire the biological functions characteristic of that class.

T cells functionally are divisible into subpopulations of effector T cells (killer T cells and T_{DTH} cells—cells involved in delayed-type hypersensitivity) and regulator cells (helper T cells and suppressor T cells). Killer T cells (T_C or CTL) directly destroy host target cells that express foreign antigens on their surfaces. T_{DTH} cells secrete messenger substances called lymphokines. Lymphokines are not antigen specific. A major function of lymphokines is to involve uncommitted cells in dealing with an antigen. Helper T (T_H) cells are essential for B and other T cells to respond to antigens. Suppressor T (T_S) cells regulate the responses of B and other T cells. Other forms of immunoregulation include feedback inhibition and the idiotype–anti-idiotype network. In feedback inhibition antibodies divert additional antigen from the antibody forming mechanism. In the idiotype–anti-idiotype network B and T cells and antibodies react with the antigen-binding sites of B and T cells.

Lymphocytes that have killer functions include the killer T cells (T_C or CTL), natural killer cells (NK cells), and K cells. NK cells are effectors of nonspecific resistance, that is, antibodies and sensitized lymphocytes need not be involved. K cells operate through an antibody-dependent mechanism. K cells have receptors for the Fc portion of the antibody molecule. Antibodies that are bound to cellular antigens via the antigen-binding site become attached to Fc receptors of the cell-destroying K cells.

The complement system is frequently involved in nonspecific resistance and in immune reactions. The biologically active factors that are serially generated when the complement system is activated facilitate the localization and destruction of foreign substances. Complement's biological roles include cell lysis, chemotaxis, opsonization, and stimulation of inflammation.

Immune states are described by the terms *innate* vs. *acquired, natural* vs. *artificial*, and *active* vs. *passive*. The level of the attained active immune state is related to such considerations as the type of immunogen, the frequency and spacing of exposure to the immunogen, the use of adjuvants, and the role of memory cells.

QUESTIONS FOR STUDY

1. Why are specificity, recognition of self antigens, and memory important features of the immune system?
2. Differentiate between immunogenicity and reactivity of antigens.
3. What is an antigenic determinant? How does it relate to an immunogen? To an antigen? To a hapten?
4. What is the function of an accessory cell? Which cell types serve as accessory cells?
5. What is the basis for naming immune lymphocytes as either B cells or T cells?
6. Differentiate between receptor Igs and secreted Igs.
7. When is an Ig properly referred to as an antibody?

8. Describe the structure of the basic Ig molecule.
9. What are the principal biological functions of the variable region and of the constant region of an Ig molecule?
10. Relate the following biological functions to a specific Ig class:
    First to be formed after antigen exposure
    Only one to cross the placenta
    Attaches to mast cells
    Serves as cell surface antigen receptor site on B lymphocytes
    Accounts for topical immunity
11. Explain how, in view of the limited genetic code, there can be so great a diversity in antibody specificities for antigens.
12. What is meant by heavy chain switch, and what is the importance of this switch?
13. How does the major histocompatibility complex relate to antigen recognition by T cells?
14. Name the effector T cells and the regulator T cells, and describe their functions.
15. What are lymphokines, and what are the functions of some of the better-known lymphokines?
16. Discuss how the immune system regulates itself.
17. Describe the lymphocytes that have killer functions.
18. Describe three ways in which the complement system contributes to immunity.
19. How do the classic and alternative pathways of the complement system differ?
20. Differentiate between innate immunity and acquired immunity; between active and passive immunity.
21. Define, describe, or explain
    MHC restriction
    Chemotaxin
    Interleukin
    Anaphylatoxin
    Opsonin
    Idiotype and anti-idiotype
    Adjuvant
    Serotherapy
    Leukoattractant
    Primary and secondary immune response
    Membrane attack complex
    Transfer factor or dialyzable leukocyte extract

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16. IMMUNODIAGNOSIS

OBJECTIVES

To cite the two features of antigen-immune product interactions that make serological testing feasible

To explain the role and the value of serological tests in diagnosis and/or identification and monitoring

To describe the procedure and the qualitative and quantitative aspects of representative serological tests; especially include agglutination, complement fixation, precipitation tests performed on support media, radioimmunoassay, fluorescent antibody, and enzyme immunoassay

To discuss monoclonal antibodies: explain how they are developed; compare monoclonal antibody preparations and conventionally produced antisera; evaluate their importance; and give examples of their diagnostic and therapeutic applications

To discuss the ABO system of red blood cell as it pertains to transfusion reactions and to discuss the RH system as it pertains to hemolytic disease of the newborn

The value of serological tests in medical microbiology
The general principles of immunodiagnosis
The Classic Serological Tests
Precipitation
Ring Test
Capillary Tube Test
Agglutination
Toxin-Antitoxin Tests
Virus Neutralization
Immune Cytolysis
Complement Fixation
Indicator System
Test System
Test Procedure
Applications of Complement Fixation
Opsonophagocytic Tests
C-reactive Protein
Quellung Reaction (Capsular Swelling)
The More Recent Immunodiagnostic Techniques
Immunodiffusion
Immunoelectrophoresis
Counterimmunoelectrophoresis
Fluorescent Antibody Technique
Direct Method
Indirect Method
Enzyme Immunoassay
Ferritin-Labeled Antibody
Radioimmunoassay
Monoclonal Antibodies Produced by Hybridomas (Fused Cells)
Hybridomas—The Source of Monoclonal Antibodies
Myeloma Cells as the Source of Vast Quantities of Immunoglobulins and "Immortality"
Spleen-Derived Lymphocytes as the Source of Antibody Specificity
Technique of Creating Hybridomas
Selection of the Hybrids to be Cloned
Advantages of Monoclonal Antibodies over Conventionally Obtained Antibodies
Applications of Monoclonal Antibodies
In Vivo Immunodiagnostic Tests
Immunology of Red Blood Cells
The ABO System
ABO System Genetics
Chemical Composition of AB Antigens
ABO Antibodies
ABO Blood Group Typing
The ABO System and Transfusion
The ABO System and Disputed Parentage
The Rh System
Determination of Rh type
Rh Isoimmunization (Alloimmunization)
Hemolytic Disease of the Newborn
Management of Rh Disease
Techniques for Determining Anti–Red Blood Cell Antibodies
Crossmatching
Coombs' Test

An important application of immune system products (antibodies and sensitized T cells) is their use in identification, diagnosis, and monitoring. The fact that the interactions between antigens and immune system products are essentially specific and that these interactions produce detectable changes make possible a great variety of valuable in vitro and in vivo tests. The subdiscipline of immunology that uses these interactions for the purposes of identification, diagnosis, and monitoring is variously entitled *serology, immunoserology, serodiagnosis, immunodiagnosis*, or *immunoidentification*. The term *serology* implies the use of serum or components thereof in the tests. Serum indeed is commonly used as a test ingredient because the most frequently used immune product, antibodies, are most accessible in serum. It should not be concluded, however, that every immunodiagnostic test involves serum.

THE VALUE OF SEROLOGICAL TESTS IN MEDICAL MICROBIOLOGY

The information obtained from serological tests is valuable for the following reasons:

1. Sometimes the only way, or the only practical way, to identify the causal agent of an infection is via serological testing. This especially is the situation with viral diseases in which the isolation of the virus is not feasible or is impractical because of cost-benefit considerations.
2. Serological tests are frequently used to confirm a diagnosis that was made on clinical grounds or by other laboratory findings.
3. It may be important to know the immune status of a person or of a population. A dentist may wish to know if he or she has antibodies to the hepatitis B virus. A woman of childbearing age may wish to know if she is immune to the rubella (German measles) virus.
4. The course or severity of a disease can be followed and the treatment or prognosis or both indicated by such determinations.
5. Sometimes it is important to know the serogroup or serotype or immunotype of a causal agent. Most species of microorganisms through mutation and selection develop strains that differ in antigenic composition. These strains also frequently differ in immunogenicity and drug susceptibility. Their identification may affect patient therapy and appropriate immunization of the population at risk.

There are many applications of serological tests outside the realm of medical microbiology: cancer detection, diagnosis of allergies, the identification and localization of tissue components (immunohistochemistry), and immunodetection in forensic pathology.

THE GENERAL PRINCIPLES OF IMMUNODIAGNOSIS

The majority of routinely employed immunodiagnostic tests involve antibodies, and so the descriptions and the tests to follow pertain almost entirely to antibodies.

How can antibodies be detected? How is it determined that an individual has antibodies to a given antigen as a result of immunization or in consequence of infection? Such determinations are valuable for diagnosis.

One of the components, either the antigen or the antibody, in a serological test is known. Thus, if the identity of an infectious agent (an antigen) recovered from a patient is unknown or is to be confirmed, or if its serotype is to be determined, it is tested against an antiserum that has a known antibody content. Conversely the antibody content of a patient's serum may be the unknown component of the test, in which case the serum is tested against known antigens. A patient with an infection, for example, after a suitable induction period has usually produced antibodies against the infecting microorganism. Blood is drawn, the serum is collected after the blood is allowed to clot, and the serum is tested against known antigen. The antigens and antisera that are used as the known component of a serological test are usually commercially available. They are stable preparations that can be kept in the refrigerator for long periods of time.

Serological tests have a qualitative aspect, and most tests also have a quantitative aspect. The qualitative aspect is to answer one of the following questions: Are there antibodies in this serum for this known antigen? Or, Does this unidentified microorganism react with this serum, which has a known antibody content? Since the antigen-antibody relationship is specific, a positive reaction usually identifies the antigen or antibody—the qualitative aspect. Not only can it be determined that the antigen and antibody interact, however, but the relative quantities of one or the other can be established; that is, a titer can be determined. This is the quantitative aspect of serological tests. Titer

can be important in discovering the causative agent of an infection, in following the course of an infectious disease, in determining the severity of a condition, and in finding out the level of protection. To determine a titer, either the antigen preparation or the serum is serially diluted in uniform increments. In a typical titration a series of 10 tubes containing doubling dilutions of the test serum is set up. A constant amount of antigen is added to each tube. The reciprocal of the highest dilution (i.e., of the last tube of the series) to give a detectable reaction in the form of a precipitation, agglutination, or other reaction is the titer. In the course of an illness it is frequently meaningful to discover that a change in titer has occurred between an early, acute-phase serum specimen and a convalescent or recovery-phase specimen.

THE CLASSIC SEROLOGICAL TESTS

Serological tests seem to have almost endless variations and applications. Over the years six kinds of antibodies have been designated: agglutinins, precipitins, antitoxins, cytolysins, opsonins, and virus-neutralizing antibodies. These terms were chosen because they are descriptive of what happens when an antigen interacts with its homologous antibody. These earlier serological test procedures were rather straightforward and uncomplicated, compared with today's refinements. In the earlier procedures straight serum was used without fractionation or conjugation. What was referred to as the antigen in many cases consisted of a complex of antigens. The tests were conducted primarily in tubes and on slides. This is not to say that such an approach is no longer used or useful. These are indeed still the basic serological tests in clinical serodiagnosis. Refinements, however, have increased the applications and the sensitivity of serological tests. The more recent techniques will be described later after the classic tests of precipitation, agglutination, toxin-antitoxin, virus neutralization, immune cytolysis, complement fixation, opsonophagocytic indexing, C-reactive protein, and quellung reaction are described.

Before antigen-antibody interactions are described it is important to know the difference between particulate test antigens and soluble test antigens. Antigens may be particulate, that is, particle-sized, as are intact bacterial cells, red blood cells, white blood cells, and particles and cells artificially coated with soluble antigens. Antigens may also be soluble, consisting of molecules in suspension.

PRECIPITATION

Two well-known techniques for demonstrating precipitating antibody are the ring test and the capillary tube test.

Ring Test

The ring test is carried out in small-diameter tubes. A column of antiserum is placed in the lower half of the tube with a capillary pipette. A column of soluble antigen is gently superimposed. Precipitation occurs at the interface in the form of a ring. In most serological tests it is the antiserum that is diluted if a titer is being determined. In the precipitation test, however, it is the antigen that is diluted since excess antigen may prevent a visible precipitate from forming even though there has been an antigen-antibody reaction. (High concentrations of either antigen or antibody may obscure a visible reaction in the earlier tubes of a titration in what is termed the *prozone phenomenon*.)

Capillary Tube Test

For the capillary tube test antigen and antiserum are sequentially drawn into a capillary tube by capillary action. Precipitates form in positive reactions. Tests that employ the capillary tube method (Fig. 16-1) are those for C-reactive protein (this test is discussed later in this chapter) and for determining the Lancefield group in the classification of streptococci.

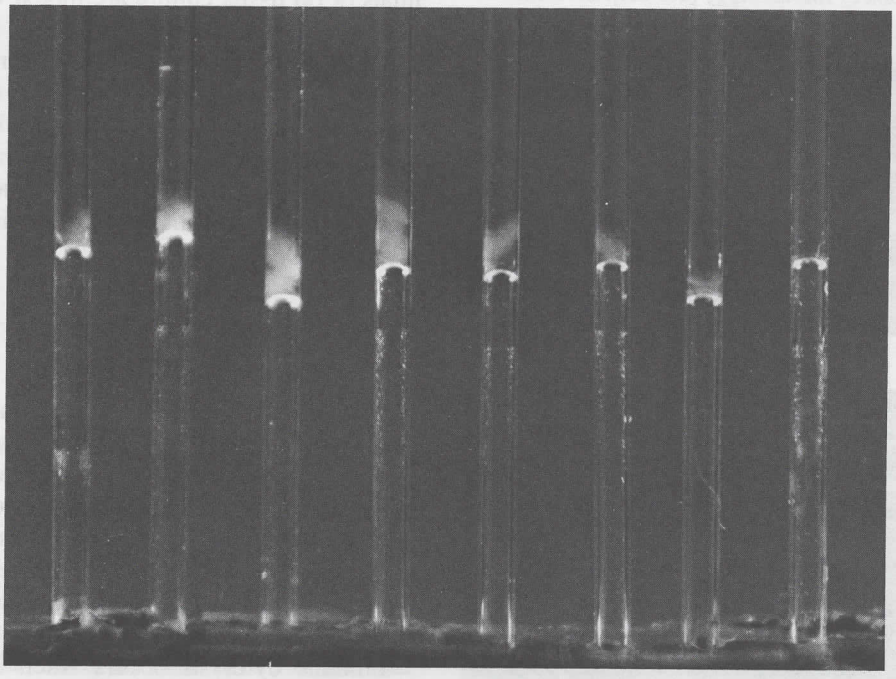


Fig. 16-1. Capillary tube precipitin test. Dilutions of soluble antigen and constant amount of specific antiserum were drawn into capillary tubes. Tubes 3 and 4 show greatest amount of precipitate. White semicircle in each tube is due to light reflection from interface where antiserum and antigen dilution meet. Final tube is negative control. (Courtesy William Krass. From J. T. Barrett, *Textbook of Immunology* [4th ed.]. St. Louis: Mosby, 1983.)

AGGLUTINATION

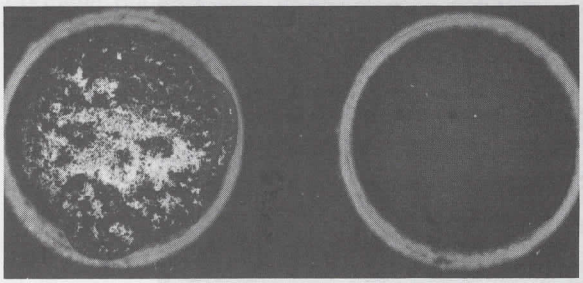
The antigen in agglutination reactions is particulate—that is, particle or cellular in size. In the presence of homologous antibody, antigens (such as intact bacterial cells or red blood cells) or uniform particles (as of latex or bentonite onto which soluble antigens have been absorbed) clump together to form visible aggregates (Fig. 16-2). Agglutination reactions are widely used for the diagnosis of certain infectious diseases and especially in human blood grouping and typing. The reaction can be carried out on a slide by mixing serum with a suspension of the antigen. Most bacterial agglutination tests are serial tube tests in which a titer is determined.

TOXIN-ANTITOXIN TESTS

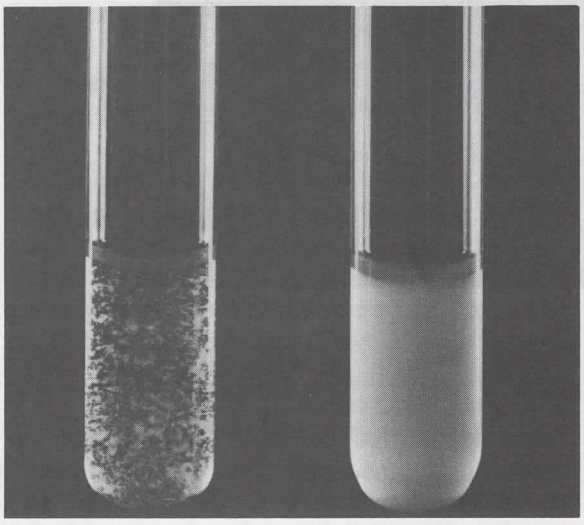
Antitoxins are generally thought of in the context of antibodies against the classic bacterial exotoxins, most especially tetanus antitoxin and diphtheria antitoxin. The effect of the antibodies in vivo is to neutralize the toxins.

Laboratory toxin-antitoxin testing generally takes the form of an in vivo neutralization test. In certain contexts it is termed a *protection test*. The indicator of whether a toxin is neutralized or not is usually an animal with a known reactivity to the toxin. Diphtheria-producing corynebacteria or diphtheria exotoxins, for example, produce an inflammatory lesion that may progress to necrosis when injected into the shaved skin of rabbits. Antitoxin can pre-

Fig. 16-2. A. Slide agglutination of bacteria is rapid method of identifying unknown bacteria with known antisera or the reverse. B. Tube agglutination can be used for same purpose. In each case positive results are seen at left and negative control at right. (From J. T. Barrett, *Textbook of Immunology* [4th ed.]. St. Louis: Mosby, 1983.)



A



B

vent the reaction. Tests of this type or related types are used to detect toxin production by *Corynebacterium* species and to assess the potency of an antitoxin preparation.

Certain toxin-antitoxin complexes also form precipitates. The Elek KL–virulence test is an in vitro precipitation test that is employed to determine whether a given *Corynebacterium* is an exotoxin-producing strain (see Fig. 22-4).

VIRUS NEUTRALIZATION

Because of their small size, viruses generally do not give readily visible reactions with their homologous antibodies. Viruses with known effects in animals or on tissue culture are mixed with serum to determine the neutralizing effect of the serum. If specific antiviral antibodies are present in the serum, the effect of the virus on the test animal or the tissue culture cells is neutralized. Other serological methods are now utilized more for virus identification and viral disease diagnosis, but neutralization tests still have some applications in the laboratory.

IMMUNE CYTOLYSIS

Antibodies and antigen, usually in combination with complement, can effect the lysis of cells. Best known of the immune cytolytic reactions are immune hemolysis and immune bacteriolysis. The respective antibodies are known as immune hemolysins and bacteriolysins. The antigen in immune cytolysis is usually the cell itself. The antigen can, however, also be artificially attached to the cell.

Immune cytolysis occurs when immunoglobulins of either class IgG or class IgM locate on the cell surface. Lesions develop in the cell surface when complement is activated, and this leads to cell lysis.

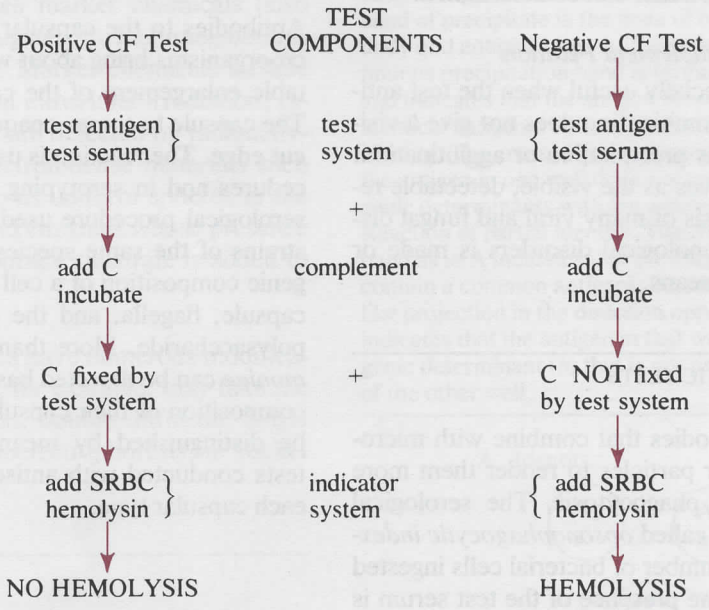
COMPLEMENT FIXATION

The phenomenon of immune cytolysis involving complement serves as the basis of an important serological test, the complement fixation (CF) test. The test has two distinct components, the indicator system and the test system. The systems vie for complement, which is one of the ingredients of the test.

Indicator System

The indicator system, commercially available, consists of sheep red blood cells (SRBC) as the anti-

Fig. 16-3. Complement fixation (CF) test. (SRBC = sheep red blood cells.)



gen and antiserum containing antibodies against SRBC. The antibody is known under the name *hemolysin* or *amboceptor*. The SRBC of the indicator system lyse when exposed to the antiserum of the indicator system in the presence of complement. Lysis is a visible reaction because the hemoglobin released from lysed red blood cells produces a pink-to-red color in the otherwise clear, uncolored cell supernatant fluid.

Test System

In the other component, the test system, either the antigen is unknown or, as is most often the case, the patient's serum is being tested for unknown antibody content. The combination of the antigen with antibody must be able to fix complement. Not all antigen-antibody combinations bind complement.

Test Procedure

The first step in the CF test is to inactivate any residual complement in the test serum (Fig. 16-3). Inactivation is achieved by heating the test serum for 30 minutes at 56°C. The order of the test procedure is as follows: The test antigen and the inactivated test serum are mixed. A measured amount of complement is added. The complement source is ordinarily fresh guinea pig serum, which is available commercially in the lyophilized form. After the mixture of the test antigen, test serum, and complement has been incubated, the indicator system is added to the test system tube. If there is antibody in the test system that is specific for the test antigen, complement is fixed or bound to the antigen-antibody combination. In a positive CF test the complement that is fixed by this combination is not available to the indicator system when it is added. Lysis therefore does not occur. In a negative CF test, in which the test antibody is not specific for the test antigen, complement is not bound by the test system and remains available to the indicator system. Lysis occurs. The test must be rigorously controlled, especially in the amount of complement that is added. Insufficient or excess complement

leads to false-positives or false-negatives. The test is not only qualitative, as described, but can also be quantitative; that is, a titer can be determined.

Applications of Complement Fixation

The CF test is especially useful when the test antigen and antibody combination does not give a visible reaction such as precipitation or agglutination. Lysis of SRBC serves as the visible, detectable reaction. The diagnosis of many viral and fungal diseases and of immunological disorders is made or confirmed by this means.

OPSONOPHAGOCYTIC TESTS

Opsonins are antibodies that combine with microbial cells and other particles to render them more readily subject to phagocytosis. The serological test for opsonins is called *opsonophagocytic indexing*. The average number of bacterial cells ingested by phagocytes in the presence of the test serum is compared with the average number ingested in the presence of the control, normal serum. This test has rarely been used, but it has been applied recently to detect defects in cellular immunity.

C-REACTIVE PROTEIN

Inflammation—either infectious or noninfectious—elicits the formation of a protein in the blood that is not an antibody. Peculiarly, it forms a precipitate with the C polysaccharide of *Streptococcus pneumoniae*. Antisera against the C-reactive protein are produced in laboratory animals and are used in the test procedure rather than the C polysaccharide of the pneumococcus. The C-reactive protein test is helpful in the diagnosis, tracing, and management of diseases such as acute rheumatic fever, cancer, infections with enteric organisms and staphylococci, and subacute bacterial endocarditis.

QUELLUNG REACTION (CAPSULAR SWELLING)

Antibodies to the capsular material of certain microorganisms bring about what appears to be a notable enlargement of the capsule (see Fig. 19-10). The capsule becomes opaque and develops a clear-cut edge. The reaction is used in identification procedures and in serotyping. *Serotyping* refers to a serological procedure used to discriminate among strains of the same species that differ in the antigenic composition of a cell component such as the capsule, flagella, and the polysaccharide of lipopolysaccharide. More than 80 strains of *S. pneumoniae* can be detected based on differences in the composition of their capsules. The differences can be distinguished by means of capsular swelling tests conducted with antisera that are specific for each capsular type.

THE MORE RECENT IMMUNODIAGNOSTIC TECHNIQUES

Refinements in testing have led to giving serological tests names that reflect the nature of the testing technique. The same kinds of antibody are involved, but the reactions are not those typical of precipitation, agglutination, cytolysis, and neutralization.

The more recent techniques are of three major types:

1. There are techniques for separating out the different antigens that may be present in the antigen material and techniques for separating out the different components of serum. The separations are performed in or on support media such as agar gel and polymeric membranes. Separation takes place under the influence of diffusion, osmosis, electrical charge, or combinations of these modalities.

2. Techniques for attaching soluble antigens or antibodies to cells (e.g., red blood cells) or to particles (e.g., latex particles) allow a visible reaction

such as lysis or agglutination to occur when antigen and antibody unite.

3. In some techniques marker chemicals (also called *labels*) are conjugated to immunoglobulins (usually) or to antigens. Marker chemicals include dyes that fluoresce upon ultraviolet irradiation, radioactive materials that are detected by radioactivity-sensing devices, electron-dense materials such as ferritin that show up as uniform spheres in the electron microscope, and enzymes whose presence is detected when appropriate substrate is added to the preparation.

Most of these techniques have numerous modifications. Gel precipitation, for example, may take the form of simple diffusion, double diffusion, single radial diffusion, immunoelectrophoresis, or rocket immunoelectrophoresis.

IMMMUNODIFFUSION

Immunodiffusion is a form of precipitation test that makes use of agar gel and other support media. Completed reactions are "fixed" in the support medium. In agar gel immunodiffusion, wells are formed in agar gel according to a predetermined pattern (Fig. 16-4). Solutions of antigen and antiserum are placed in the wells. Antigens and antibodies diffuse toward each other in a radial pattern. Where homologous antibody reacts in proper proportions with antigen, lines of precipitate form within the agar.

This method has many good features. One is the stability of the precipitate; that is, once the precipitate is formed, it will remain fixed in that position in the agar over a long period of time. In addition, more than one antigen or antibody can be tested because the different antigenic molecules in the solution diffuse and aggregate on the basis of their size, configuration, and other properties. And further, unknown antigens can be identified when known control antigens in a neighboring well form continuous lines of precipitate with the unknown. A continuous line (fusion) indicates identity; crossed lines, nonidentity. The major disadvantage of the

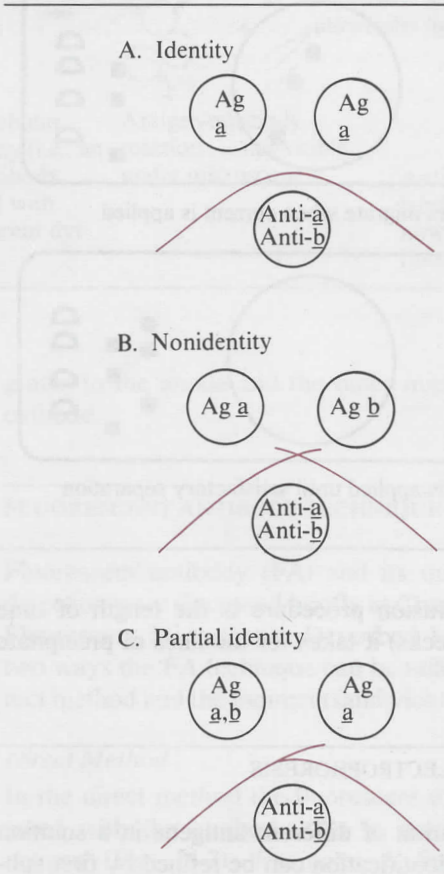
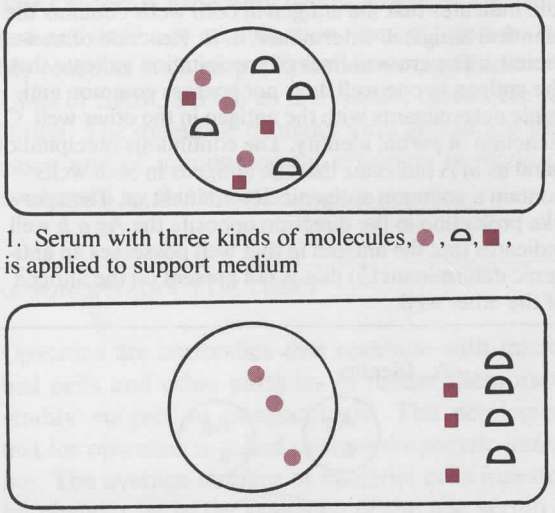


Fig. 16-4. Immunodiffusion in agar gel. Antigen-antibody recognition results in a readily visible, opaque band of precipitate in the zone of equivalence of antibody and antigen (*Ag*). A. Reaction of identity. The continuous precipitation band is termed a *line of identity* and indicates that the antigen in both wells contains the identical antigenic determinant *a*. B. Reaction of nonidentity. The crossed lines of precipitation indicate that the antigen in one well does not possess common antigenic determinants with the antigen in the other well. C. Reaction of partial identity. The continuous precipitation band as in A indicates that the antigens in both wells contain a common antigenic determinant, *a*. The spur-like projection in the direction opposite the Ag *a,b* well indicates that the antigen in that well possesses an antigenic determinant (*b*) that is not present on the antigen of the other well.

Fig. 16-5. Electrophoresis. One method of separating molecules of particles in a solution is to apply current to the support medium. The charged molecules or particles move toward the oppositely charged electrode.



1. Serum with three kinds of molecules, ●, ▱, ■, is applied to support medium

2. Molecules migrate when current is applied

3. Current is applied until satisfactory separation is obtained

immunodiffusion procedure is the length of time (days to weeks) it takes for the lines of precipitate to develop.

IMMUNOELECTROPHORESIS

The separation of different antigens in a solution and their identification can be refined by first subjecting the solutions within a support medium to electrical current (electrophoresis) (Fig. 16-5). On the basis of their charges, molecular size, and other properties, the various antigens or proteins of a serum are separated in or on a support medium such as thin layers of agar gel, cellulose acetate, or starch. Electrophoresis is combined with immunodiffusion in immunoelectrophoresis. After or during electrophoretic separation, troughs for antiserum (or other methods of application) are placed

Fig. 16-6. Immunoelectrophoresis of human serum. A strip of agarose that contains electrophoretically resolved human serum proteins is placed along the bottom edge, and molten agarose that contains goat antiserum is poured over the plate. When the plate has gelled, the antigens in the human serum are forced into the antibody-containing gel layer by electrophoresis. Thirty-seven precipitation zones can be seen. Some of the antigens identified in the human serum are albumin (*A*), ceruloplasmin (*D*), hemopexin (*H*), and gamma globulin (*L*). (From H. Michin Clarke, in C. Z. Williams and M. W. Chase [Eds.], *Methods in Immunology and Immunochemistry*, Vol. 3. New York: Academic, 1971.)

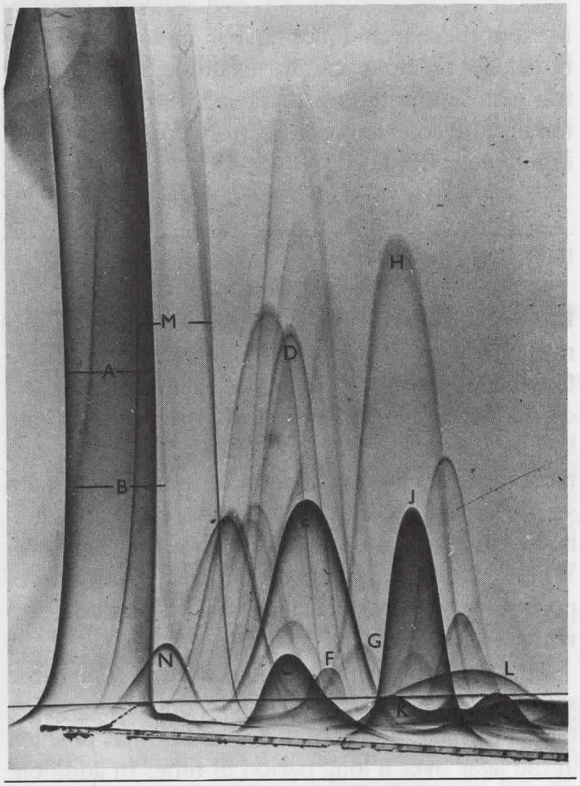
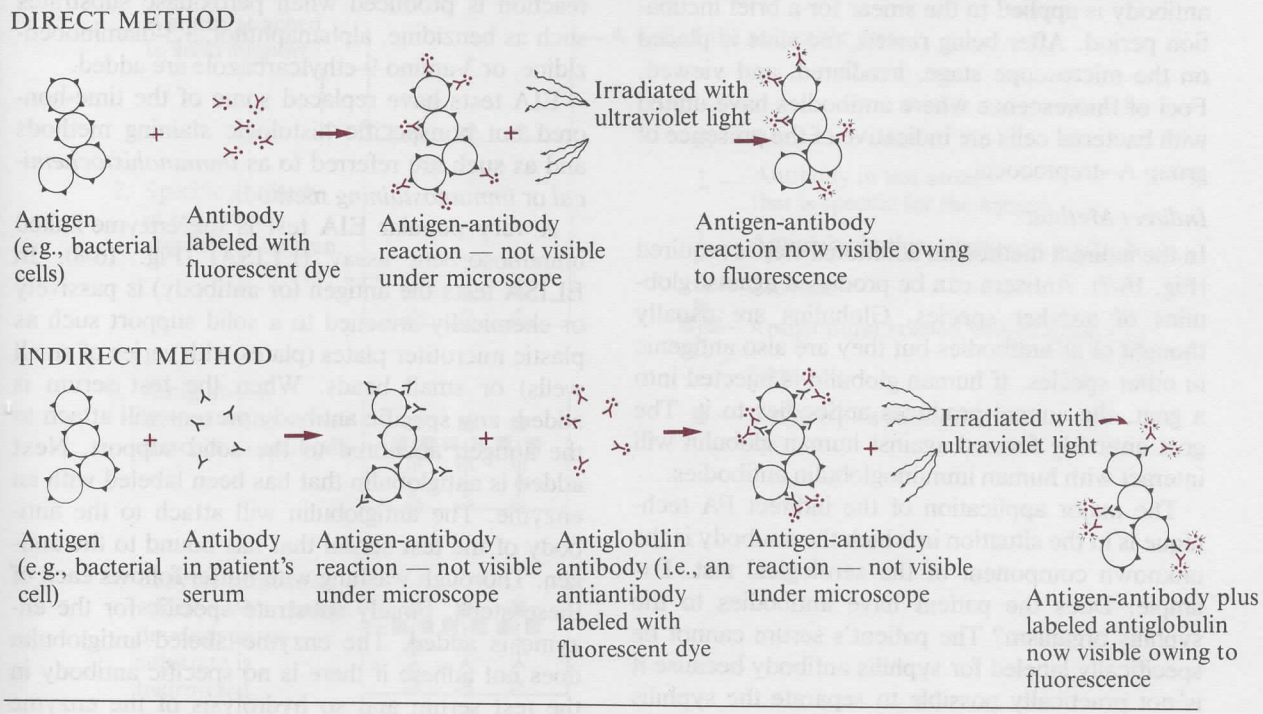


Fig. 16-7. Fluorescent antibody techniques.



parallel to the lines of migration of the electrophoretic solution. Arcs of antigen-antibody precipitate form. Human serum, as antigen, has been extensively investigated by this technique. As many as 37 zones of precipitate, representing different proteins or subgroups of a protein class of the serum, have been distinguished (Fig. 16-6).

COUNTERIMMUNOELECTROPHORESIS

Counterimmunoelectrophoresis is so named because the antigen and antibody are made to flow toward each other in opposite directions. Precipitation occurs where they meet in optimal proportions. The supporting gel is at a pH that is between the isoelectric pH of the antigen and that of the antibody (electrophoretic migration does not occur at the isoelectric pH) so that one of the reactants migrates to the anode and the other migrates to the cathode.

FLUORESCENT ANTIBODY TECHNIQUE

Fluorescent antibody (FA) and its microscopical detection were discussed briefly in Chapter 3 under Fluorescent Microscope. Described here are the two ways the FA technique can be utilized: the direct method and the indirect (sandwich) method.

Direct Method

In the direct method the fluorescent dye is conjugated with the antibody that is specific for the antigen (Fig. 16-7). Example: A fluorochrome-antibody conjugate that is specific for group A streptococci can be purchased or prepared. In a test situation a throat specimen suspected of con-

taining group A streptococci is affixed to a slide. The antiserum containing the fluorescent anti-A antibody is applied to the smear for a brief incubation period. After being rinsed, the slide is placed on the microscope stage, irradiated, and viewed. Foci of fluorescence where antibodies have united with bacterial cells are indicative of the presence of group A streptococci.

Indirect Method

In the indirect method an additional step is required (Fig. 16-7). Antisera can be produced against globulins of another species. Globulins are usually thought of as antibodies but they are also antigenic in other species. If human globulin is injected into a goat, the animal produces antibodies to it. The goat antibody formed against human globulin will interact with human immunoglobulin antibodies.

The major application of the indirect FA technique is in the situation in which the antibody is the unknown component of the serological test. Example: Does the patient have antibodies to the syphilis organism? The patient's serum cannot be specifically labeled for syphilis antibody because it is not practically possible to separate the syphilis antibody immunoglobulins from the other globulins of the serum. Instead, the unlabeled serum can be reacted with the syphilis antigen on the slide. If there are antibodies to the antigen, the reaction is not visible at this time. When the labeled goat anti–human globulin is added after the patient's serum is rinsed off, it will attach to the syphilis antibody and upon irradiation there will be foci of fluorescence. Fluorescence usually occurs only if there is antibody from the patient attached to the antigen on the slide.

ENZYME IMMUNOASSAY

A popular type of label employs enzymes that have been conjugated with antibody. Enzyme immunoassay (EIA) tests are highly specific, versatile, accurate, reproducible, and relatively inexpensive. When the antibody with the attached enzyme reacts with its specific antigen the antigen or the antigen location or both can be detected by adding the substrate that the enzyme attacks. In a positive immunoperoxidase test, for example, a colored reaction is produced when peroxidase substrates such as benzidine, alphanaphthol, 3,3-diaminobenzidine, or 3-amino-9-ethylcarbazole are added.

EIA tests have replaced some of the time-honored but nonspecific histologic staining methods and as such are referred to as *immunohistochemical* or *immunostaining* methods.

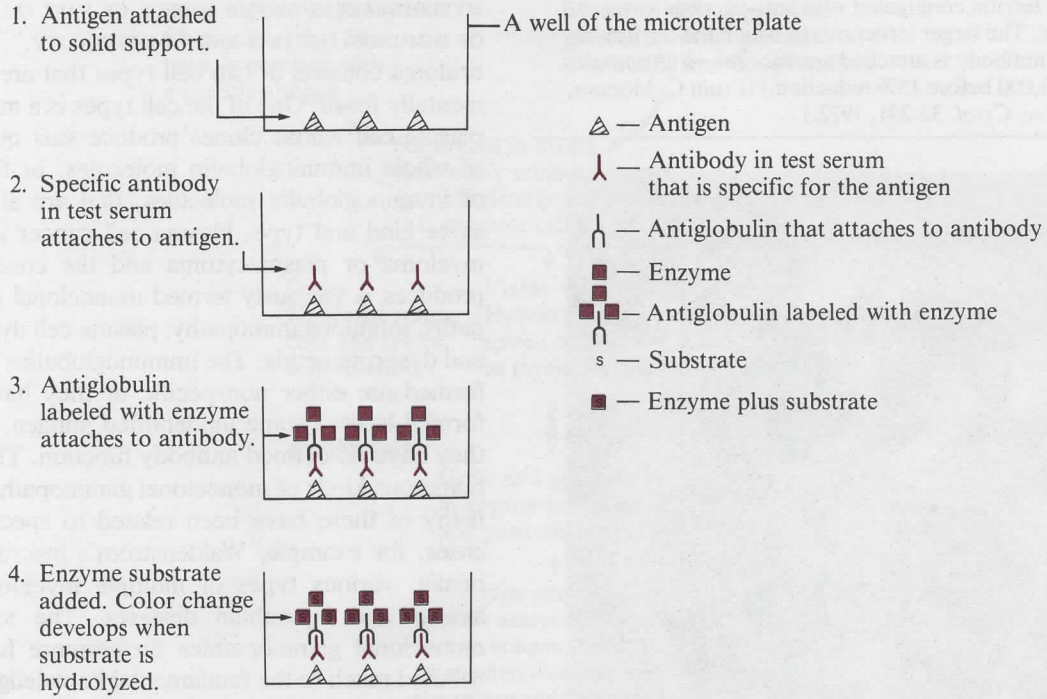
A very popular EIA test is the enzyme-linked immunosorbent assay (ELISA) (Fig. 16-8). In ELISA tests the antigen (or antibody) is passively or chemically attached to a solid support such as plastic microtiter plates (plates with series of small wells) or small beads. When the test serum is added, any specific antibody present will attach to the antigen anchored to the solid support. Next added is antiglobulin that has been labeled with an enzyme. The antiglobulin will attach to the antibody of the test serum that has bound to the antigen. Thorough washing with buffer follows each of these steps. Finally substrate specific for the enzyme is added. The enzyme-labeled antiglobulin does not adhere if there is no specific antibody in the test serum and so hydrolysis of the enzyme substrate with the accompanying color change does not occur. Color development is either detected visually or it is spectrophotometrically measured.

EIA techniques offer several advantages over the FA technique and radioimmunoassay: The conventional light microscope is used for EIA, whereas the FA method requires an ultraviolet light source; the EIA preparations are permanent, whereas in the FA technique the fluorescence vanishes after a short time; fewer nonspecific reactions reportedly occur with the EIA than with the FA technique; and the unstable reagents, expensive equipment, and safety and licensing problems of radioimmunoassay are avoided.

FERRITIN-LABELED ANTIBODY

Globulins can be conjugated with ferritin, an iron-containing protein (Fig. 16-9). Antibodies so la-

Fig. 16-8. Positive ELISA test.



beled are used to localize antigens in electron microscope studies. Ferritin molecules are electron-dense and have the characteristic of showing up as very small, uniform, darkened spheres in electron micrographs.

RADIOIMMUNOASSAY

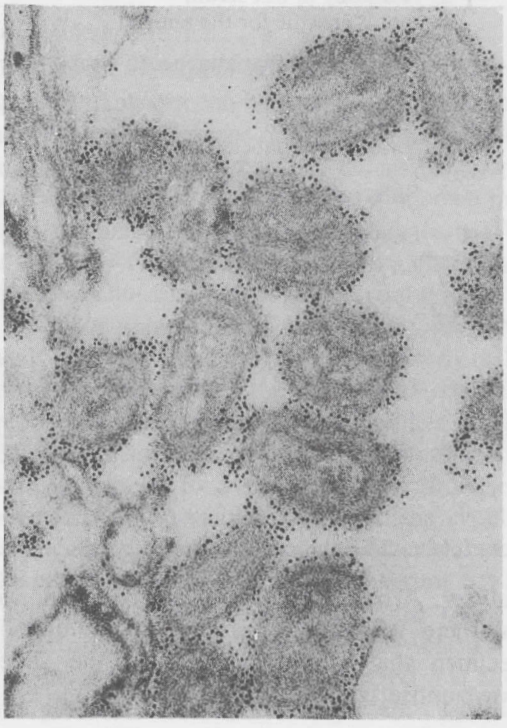
A microtechnique of great sensitivity uses antigens that are labeled with radioactive iodine. The unknown, unlabeled antigen is exposed to antibody that is specific for a known antigen. The known, labeled antigen of a certain concentration is subsequently added to the same tube. After a suitable incubation period an anti–gamma globulin is added that attaches to the specific antibody. This action causes precipitation of the specific antibody and of the antigen to which the specific antibody is attached. The precipitate is counted in a radioisotope analyzer. A reduced count or no count indicates that there was antigen present in the unknown specimen that is homologous for the specific known antiserum.

MONOCLONAL ANTIBODIES PRODUCED BY HYBRIDOMAS (FUSED CELLS)

A recent, immensely important technique that lends a high degree of specificity, uniformity, and rapidity to immunodiagnosis involves applications of monoclonal antibodies. Monoclonal antibodies are produced in vitro (also in vivo, as described later) by a hybrid cell. The hybrid cell results from the fusion of a cancerous plasma cell (called a myeloma cell) and an antibody-producing lymphocyte obtained from the spleen.

Historically the hybridization of somatic cells originated in the 1960s. J. W. Littlefield in 1964 was

Fig. 16-9. Electron micrograph of ferritin-conjugated antibody. The small, uniform, dark spheres are electron-dense ferritin conjugated with anti–vaccinia virus antibodies. The larger structures to which the ferritin-labeled antibody is attached are vaccinia virus particles. (×126,000 before 15% reduction.) (From C. Morgan, *Int. Rev. Cytol.* 32:291, 1972.)



able to nurture hybridized cells in vitro by selectively poisoning the parental cell lines. C. Milstein and G. Kohler, building on these pioneering studies, fused myeloma cells and spleen cells. The resulting hybridoma produced the first monoclonal antibody of predefined specificity. This monumental biotechnical advance, for which Milstein and Köhler were awarded the Nobel prize, has launched a multitude of actual and anticipated applications not only for immunodiagnosis, which is our interest here, but also for immunotherapy.

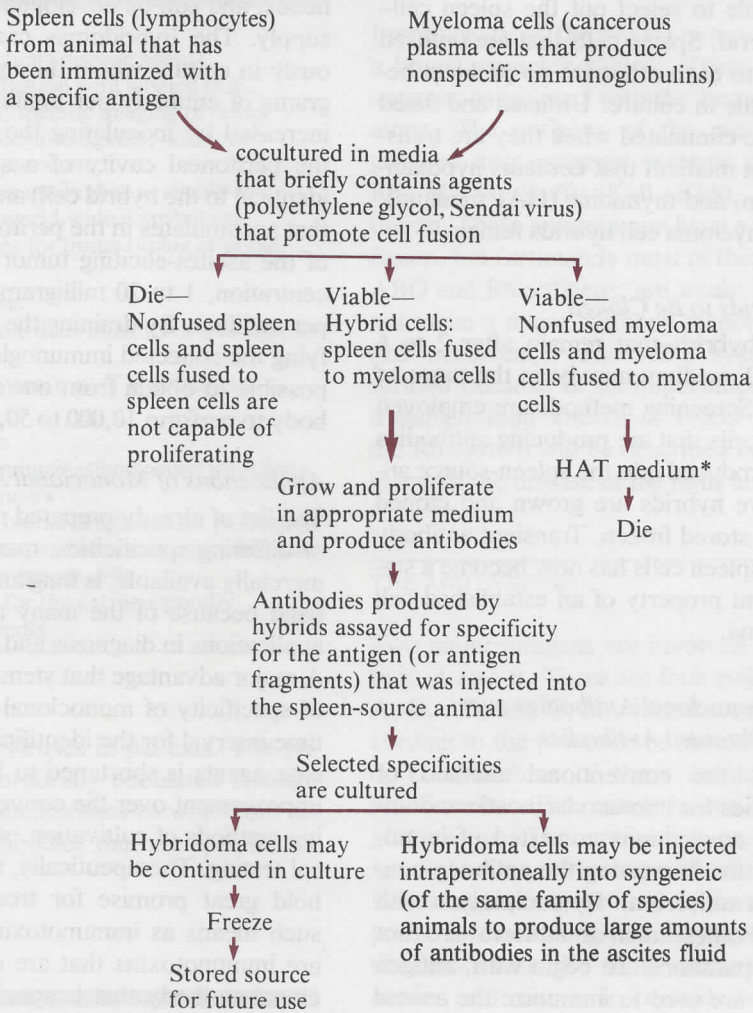
Hybridomas—The Source of Monoclonal Antibodies

MYELOMA CELLS AS THE SOURCE OF VAST QUANTITIES OF IMMUNOGLOBULINS AND "IMMORTALITY." The hybridoma consists of two cell types that are experimentally fused. One of the cell types is a malignant plasma cell whose clones produce vast quantities of whole immunoglobulin molecules, or fractions of immunoglobulin molecules, that are all of the same kind and type. Plasma cell cancer is called myeloma or plasmacytoma and the condition it produces is variously termed monoclonal gammopathy, immunogammopathy, plasma cell dyscrasia, and dysproteinemia. The immunoglobulins that are formed are either nonspecific or they have been formed against some unidentified antigen, that is, they have no defined antibody function. There are numerous kinds of monoclonal gammopathies, and many of these have been related to specific diseases, for example, Waldenström's macroglobulinemia, various types of multiple myeloma, and heavy- and light-chain diseases. The study of monoclonal gammopathies furthermore has contributed much to the fundamental knowledge about the immune system. The monostructural immunoglobulins produced by myeloma cell strains have been especially important in the determination of the basic structure of the immunoglobulin molecule and in distinguishing the five immunoglobulin classes.

The contribution of the myeloma cell to the hybrid resides in its vigorous, continuous growth both in vitro and in vivo and in the virtually endless supply of immunoglobulins it is capable of producing. The permanent and robust growth has caused some to say that the myeloma half of the hybrid imparts "immortality."

SPLEEN-DERIVED LYMPHOCYTES AS THE SOURCE OF ANTIBODY SPECIFICITY. The other half of the hybridoma cell is usually obtained from the spleen of an animal that has been deliberately immunized with a given antigen. The contribution of spleen cells are lymphocytes that are producing antibodies of a single specificity. Lymphocytes in culture are incapable

Fig. 16-10. Hybridoma technique.



*Unfused myeloma cells and myeloma-myeloma hybrids cannot survive in HAT (hypoxanthine, aminopterin, thymidine) medium because myeloma cells are selected that lack the enzyme HPRT (hypoxanthine phosphoribosyl transferase).

of sustained division so it is not possible to develop continuous cell lines of lymphocytes that are producing specific antibodies.

TECHNIQUE OF CREATING HYBRIDOMAS. Since only a small proportion of the total spleen cell population is responding to the antigen it is necessary to bring together a large number of spleen (1 to 3 $\times$ 10^8) and myeloma (1 to 10 $\times$ 10^7) cells to increase the likelihood of contact between antibody-producing cells and myeloma cells. Fusion occurs spontaneously, but it can be enhanced by the addition of such agents as polyethylene glycol or Sendai virus (Fig. 16-10). Fusion takes place between spleen cell and

spleen cell, myeloma cell and myeloma cell, and spleen cell and myeloma cell. It therefore becomes essential to be able to select out the spleen cell–myeloma cell hybrid. Spleen cells that are unfused or that are fused to each other are no problem because they soon die in culture. Unfused and fused myeloma cells are eliminated when they are transferred to a growth medium that contains hypoxanthine, aminopterin, and thymidine (HAT medium). The spleen cell–myeloma cell hybrids remain.

Selection of Hybrids to Be Cloned

The number of hybrids that remain after 2 to 6 weeks in the HAT medium may be in the range of 150 to 500 cells. Screening methods are employed to select out the cells that are producing antibodies to the antigen introduced into the spleen-source animal. The positive hybrids are grown and cloned and may then be stored frozen. Transient antibody secretion by the spleen cells has now become a stable and permanent property of an established cell line, the hybridoma.

Advantages of Monoclonal Antibodies over Conventionally Obtained Antibodies

Over the years the conventional method of obtaining antibodies for immunodiagnostic and immunotherapeutic applications consisted of immunizing an animal and harvesting the antibody-containing serum (an antiserum). By comparison with monoclonal antibodies, animal antisera are not very "clean" preparations. To begin with, antigen preparations that are used to immunize the animal are usually impure. The antiserum from the animal therefore is a very heterogeneous mixture of antibody specificities. Even though each B cell makes only one specific antibody, there are hundreds of B cells making different antibodies and their products are mixed in the serum. The "specificity" of a given antiserum, for the most part, is determined by those antibodies that are present in highest concentration; those of lower activity are usually masked.

Monoclonal antibodies by contrast have highly defined characteristics. They have an extremely precise specificity (in fact sometimes too narrow for practicality) and uniform avidity, are homogeneous, and can be developed in virtually unlimited supply. The hybridomas can be grown continuously in culture where they yield 10 to 100 micrograms of antibody per milliliter. The yield can be increased by inoculating the hybridoma cells into the peritoneal cavity of a syngeneic (genetically identical to the hybrid cell) animal. The serous fluid that accumulates in the peritoneal cavity as a result of the ascites-eliciting tumor contains a high concentration, 1 to 20 milligrams, of immunoglobulin per milliliter. By draining the ascites fluid and purifying the collected immunoglobulin it is sometimes possible to obtain from one mouse sufficient antibody to perform 10,000 to 50,000 diagnostic tests.

Applications of Monoclonal Antibodies

The list of already prepared monoclonal antibodies of differing specificities, many of which are commercially available, is long and shall continue to expand because of the many actual and anticipated applications in diagnosis and therapy (Table 16-1).* A major advantage that stems from the high degree of specificity of monoclonal antibodies is that the time interval for the identification of infectious disease agents is shortened to 15–20 minutes, a great improvement over the conventional, time-consuming methods of cultivation, staining, and biochemical testing. Therapeutically, monoclonal antibodies hold great promise for treating cancers through such means as immunotoxins. Being investigated are immunotoxins that are composed of a monoclonal antibody that is specific for antigens of the patient's cancer cells and toxins that are covalently bound to the antibody. The toxic polypeptide A chain of the plant toxin ricin is a candidate toxin for this type of cancer immunotherapy. The antibody specifically attaches to the cancer cell, and the attached toxin enters the cancer cell and poisons it. Another interesting application is in the removal or

*A Hybridoma Data Bank located at the American Type Collection Headquarters in Rockville, MD, was instituted in September 1984. The bank will serve as a source for locating hybridomas of given specificities and will avoid duplication of effort.

Table 16-1. Actual and Anticipated Applications of Monoclonal Antibodies

DIAGNOSTIC APPLICATIONS
In vitro diagnosis of infectious disease agents
In vivo diagnosis or localization with labeled (e.g., radioactive) antibodies; specific imaging of tumor metastases, organs, infectious agents, hormone receptors, neuron receptors, etc.
As standardized diagnostic antibodies available in limitless quantities for world-wide distribution
As standardized antibodies for tissue typing or organ transplants
INVESTIGATIVE APPLICATIONS
Analyses of single antigenic determinants of a complex antigen
Discernment of cell subtypes, e.g., T lymphocyte subtypes
THERAPEUTIC APPLICATIONS
Highly specific passive immunization against infectious agents, toxic drugs, cancers
As carriers of covalently bound drugs against pathogens
As carriers of toxic substances (immunotoxins) that can be precisely focused on cancer cells
As toxin or virus captors for the extracorporally circulated blood of patients

reduction of toxins or viruses in patients. The patient's blood is extracorporally circulated through hollow fibers or over membranes to which are attached monoclonal antibodies that are specific for the toxins or viruses.

IN VIVO IMMUNODIAGNOSTIC TESTS

In vivo tests are generally not called serological tests unless they are neutralization tests. Instead, in vivo tests are variously named after the mode of application of the test antigen, the disease in question, or the person who devised the test: skin test, histoplasmin test, tuberculosis skin test, Schick test, Frei test. Most in vivo tests are qualitative tests, with only one dilution of antigen being used. The size and intensity of the reaction do, however, lend a quantitative aspect.

IMMUNOLOGY OF RED BLOOD CELLS

Immunology of the blood, *immunohematology*, is a broad, complex subject. There are hundreds of antigens associated with the human red blood cell alone. The antigens of the red blood cells are grouped into antigenic systems or factors: ABO, Rh, MNSs, Duffy, Kell, Kidd, Lewis, and Lutheran. These antigens can have a bearing on transfusion, but fortunately most of them, except for the ABO and Rh antigens, are weak: Most of them do not incite a major antibody response or are of limited occurrence. The ABO system will be described because of its importance in that type of transplantation known as blood transfusion, and the Rh system will be described because of its role in hemolytic disease of the fetus and newborn.

THE ABO SYSTEM

Two major antigens are involved in the ABO system: A and B. There are four major blood groups, A, B, AB, and O, in which humans are placed according to the presence or absence A or B antigen or combinations of those antigens on their red blood cells. The antigens are termed *hemagglutinogens*. The antibodies are referred to as *hemagglutinins*. More specifically, they are called *isohemagglutinogens* and *isohemagglutinins*, the prefix *iso-* indicating that they are antigens and antibodies within the same species (as opposed to *hetero-* and *auto-*). To coincide with the terminology used in tissue and organ transplantation the prefix *allo-* is now replacing *iso-*. *Iso-* signifies an exactly alike genetic makeup and therefore exactly alike cell and tissue antigens of members of the same species. *Allo-* signifies members of the same species but with a different genetic makeup. In the human population only identical (uniovular) twins are genetically identical.

ABO System Genetics

The antigens A and B are genetically determined codominants. Transmission follows mendelian in-

Table 16-2. ABO Blood Group System

ABO phenotypes	Serum antibodies	Percent of population	Possible genotypes	Possible phenotypes when mated with AA	AO	BB	BO	OO	AB
A	Anti-B	42	AA	A	A	AB	A, AB	A	A, AB
			AO	A	A, O	B, AB	A, B, AB, O	A, O	A, B, AB
B	Anti-A	10	BB	AB	B, AB	B	B	B	B, AB
			BO	A, AB	A, B, AB, O	B	B, O	B, O	A, B, AB
O	Anti-A, Anti-B	44	OO	A	A, O	B	B, O	O	A, B
AB	None	4	AB	A, AB	AB, A, B	B, AB	AB, A, B	A, B	AB, A, B

heritance patterns. The blood group that a person belongs to which is determined by slide or tube agglutination test procedures, represents the phenotype. The genotypes of groups O and AB are automatically known when the phenotype is known. The genotype of a group O person is OO; of group AB, it is AB. Group AB individuals can transmit the A or the B determinant to the offspring; group O transmits neither. Group A and group B individuals may be genotypically homozygous (AA, BB) or genotypically heterozygous (AO, BO). Table 16-2 shows selected parental phenotypes and genotypes and possible offspring types. There are subgroups to the A and B antigens of which antigenic variants A_1 and A_2 are the most frequently encountered.

Chemical Composition of AB Antigens

Chemically the blood group substances are mucopeptides (85 percent carbohydrate, 15 percent peptide). The determinant that is associated with the group A mucopeptide is alpha-*N*-acetylgalactosaminyl-galactose. The group B determinant is the alpha-D-galactosyl-galactose. These substances appear not only on the red blood cell but also commonly in body cells and fluids.

ABO Antibodies

Antibodies (isohemagglutinins or allohemagglutinins) to the A and B antigens are "naturally" occurring. They develop spontaneously without a known stimulus. Thus, an individual who belongs to blood group A automatically has anti-B antibodies, one in group B has anti-A antibodies, one in group AB has no antibodies to the A and B antigens, and one in group O has anti-A and anti-B antibodies. Why the antibodies are naturally occurring is a subject for speculation. The chemical groupings of the A and B antigens are very common in nature. People who have the genetically determined antigens probably mark them as "self" antigens during fetal development and do not produce the corresponding antibodies. Those who do not possess the genetically determined antigens in utero produce antibodies when their immune system becomes competent and contacts the widespread A and B antigens.

ABO Blood Group Typing

Commercially prepared antisera are used in a slide or tube agglutination procedure to determine the ABO phenotype of the patient's red blood cells.

The ABO System and Transfusion

The major consideration in blood transfusion is based on the recipient's antibodies vs. the donor's cells. Incorrectly matched cells are destroyed by the recipient's antibodies. The donor's antibodies, unless a large amount of blood is being transfused, are diluted by the recipient's volume of blood. Blood group O individuals are termed *universal donors* because their red cells lack the A and B antigens and so, even if the recipient has antibodies, no reaction normally occurs in respect to the ABO system. AB individuals, lacking anti-A and anti-B

antibodies, are termed *universal recipients*. In routine practice people are transfused as far as possible with blood of their own ABO groups and Rh compatibility. It is not safe to transfuse a patient solely on the basis of information obtained in the routine ABO and Rh typing procedures. Those are merely preliminary tests that determine the blood group of the cells and of the patient's serum. Additional safeguards must be employed to detect incompatibilities between the prospective donor's cells and the recipient's serum. Other testing techniques that involve the recipient's serum are described after the discussion of the Rh system.

The ABO System and Disputed Parentage

The ABO blood group system may be of value in determining disputed parentage. It is sometimes possible to exclude an individual accused of fathering a child on the basis of the ABO blood group testing. Thus, an O male could not father an AB child. This technique does not, however, prove that a male is the father just because the blood group of the child is compatible with the blood group of the putative father.

THE Rh SYSTEM

The Rh system is even more complex than the ABO system because of the many antigens involved. The Rh system, second only to the ABO system in importance, is particularly crucial in the situation of an Rh-positive fetus borne by an Rh-negative mother. Notable differences from the ABO system are that the Rh antibodies belong to immunoglobulin class IgG (they cross the placenta) and that Rh antibodies are not naturally occurring. For Rh antibodies to develop, there has to be an opportunity for antigenization by the Rh factor or factors.

Determination of Rh Type

In preliminary general testing, individuals are designated Rh-positive if their red blood cells have Rh factor D. They are Rh-negative if they lack that factor, symbolized by the lowercase letter *d*. An individual can also be Rh-positive on the basis of other Rh factors, C and E, but these are of much lower incidence. About 85 percent of the white population is Rh-positive. Persons who are Rh-positive are homozygous (D/D) or heterozygous (D/d) for the major factor. Thus, a prospective father who phenotypically is Rh-positive may be heterozygous (D/d), and in that case there is a 50 percent chance that the child will be Rh-negative if the mother is Rh-negative.

Rh Isoimmunization (Alloimmunization)

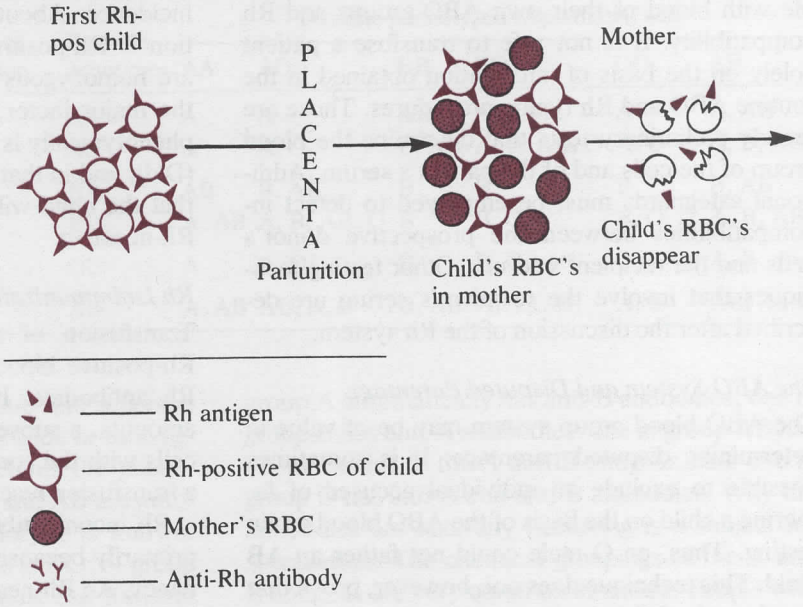
Transfusion of an Rh-negative individual with Rh-positive blood leads to the production of anti-Rh antibodies. If these are formed in sufficient amounts, a subsequent transfusion with red blood cells with the corresponding Rh antigen will cause a transfusion reaction.

Rh incompatibility problems, however, develop primarily because of isoimmunization during pregnancy. An Rh-negative mother carrying an Rh-positive fetus produces anti-Rh antibodies when the fetus's Rh-positive cells enter the mother's circulation (Fig. 16-11). The best opportunity for the fetus's cells to enter the mother is during the perinatal period when "major bleeds" occur. Late in pregnancy the epithelium that covers the placental villi atrophies, rendering the villi more fractious and therefore more vulnerable to penetration by the fetal cells. The antigenization by the fetal red blood cells stimulates the production of anti-Rh antibodies by the mother. In a blood group O mother the child's red blood cells can also evoke the production of additional anti-A or anti-B antibodies if the child is of a non-O genotype. An important distinction: The naturally occurring anti-A and anti-B antibodies are of the IgM class and do not cross the placenta.

Hemolytic Disease of the Newborn

The titer of anti-Rh antibodies generally does not reach a level high enough to be damaging to fetal red blood cells until about the third Rh-incompatibility pregnancy. The pathology results when the mother's antibodies cause the lysis of the child's red blood cells. The degree of lysis is governed by

Fig. 16-11. Development of Rh hemolytic disease of the newborn. (RBC = red blood cell.)



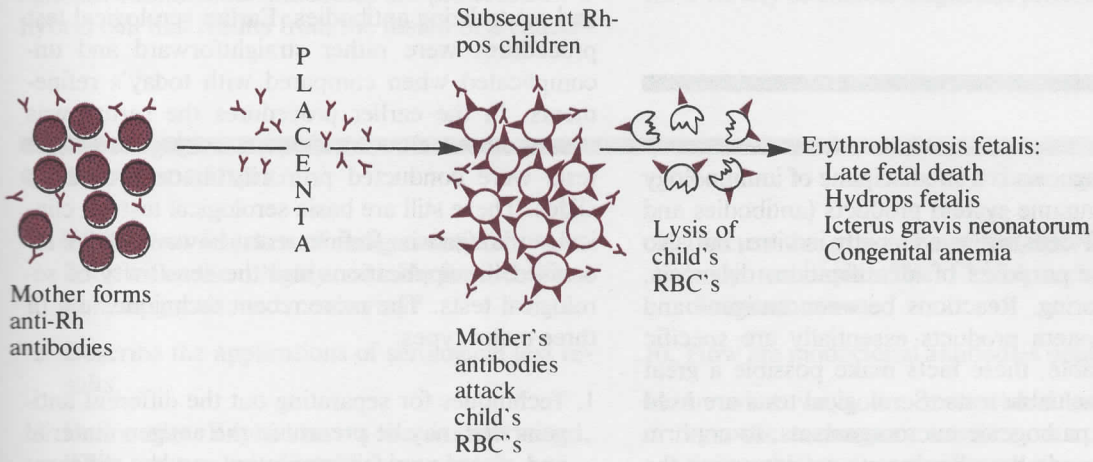
the titer of the mother's antibodies. In hemolytic disease of the fetus and newborn (erythroblastosis fetalis and erythroblastosis neonatorum) the maternal antibodies destroy red blood cells of the child late in fetal life or in the neonatal period (Fig. 16-11). Immature, nucleated red blood cells (erythroblasts) increase in number to replace the lysed red blood cells. Three clinical conditions (listed in decreasing order of severity) can follow: hydrops fetalis (extensive pouring out of blood fluid into the tissues), icterus gravis neonatorum (jaundice), and congenital anemia.

Management of Rh Disease

1. The anti-Rh antibody level of women can be monitored during pregnancy if an Rh incompatibility exists between the mother and the father, or when there has been previous hemolytic disease in offspring of multiparous women. Serious fetal disease is not likely to occur if the mother's titer is less than 1:8. A good indication of impending disease is discerned by Coombs' test (see the following section), carried out on cord blood. Serum bilirubin levels in the child reflect the severity of the child's condition.

2. If an exchange transfusion of blood, which is a slow-dilution procedure, is required for the newborn child, the Rh-positive child is transfused with Rh-negative blood. The mother's anti-Rh antibodies in the child's system would destroy transfused Rh-positive red blood cells.

3. It is possible to prevent or to minimize the isoimmunization of a mother who does not already have Rh antibodies by injecting anti-Rh antibodies into the mother within 72 hours after the birth of an Rh-positive child. The anti-Rh antibodies are in the form of a gamma globulin preparation called *RhIG*. The passive immunization suppresses the antigenization by the fetal cells in the postpartum period. Preventing antibody formation at this juncture forestalls the development of an Rh incompatibility problem in the next pregnancy.



TECHNIQUES FOR DETERMINING ANTI-RED BLOOD CELL ANTIBODIES

It is important to be able to detect incompatibilities in vitro for monitoring purposes and to prevent transfusion reactions. The antibodies, either the naturally occurring ones or those that result from isoimmunization or idiosyncrasies (self peculiarities), are not all of the same type. Several techniques are therefore employed so as not to overlook the presence of incompatible antibodies. Positive reactions are in the form of hemagglutination or hemolysis.

Crossmatching

After routine ABO and Rh slide and tube tests are carried out with commercial antisera, crossmatches are performed before transfusion. Crossmatching detects incompatibilities that are not discovered in the routine typing and picks up typing procedure errors. In the major crossmatch the donor's cells are tested against the recipient's serum. The donor's serum is tested against the recipient's cells in the minor crossmatch.

Coombs' Test

Antibodies do not always give a detectable reaction in the crossmatching procedure. Some antibodies, called *incomplete* or *blocking antibodies*, even though they attach to the red blood cells, do not give the visible reaction of hemagglutination or hemolysis. The principle of Coombs' test lies in providing a divalent immunoglobulin bridge between the monovalent antibody molecules. The immunoglobulin bridge is an antibody against human globulin. It is produced by injecting human globulin into goats or rabbits. The animal forms antibodies to the human globulin. To understand Coombs' test, envision the incomplete antibodies attached to red blood cells. A visible reaction does not occur. When the animal serum containing the bridging antibodies is added to such coated cells, the divalent

animal molecule joins two human immunoglobulin molecules together, leading to the visible reaction.

SUMMARY

Immunodiagnosis is a subdiscipline of immunology in which immune system products (antibodies and sensitized T cells) are used mostly in vitro, but also in vivo, for purposes of identification, diagnosis, and monitoring. Reactions between antigens and immune system products essentially are specific and detectable; these facts make possible a great variety of valuable tests. Serological tests are used to identify pathogenic microorganisms, to confirm diagnoses made by other means, to determine the immune status of an individual or of a population, to follow the course of a disease, and to type or group members of the same species of microorganism on the basis of antigenic differences.

Serological tests may be as simple as placing an antigen and serum on a slide or in a single tube and observing whether a reaction occurs. In many instances, however, a single concentration of both components is not satisfactory because a single positive reaction with little or no dilution may represent former disease or cross reactivity, or a positive reaction may not be obtained because a disproportionately high concentration of antigen or antibody may prevent the development of visible aggregates. Serological tests frequently have a quantitative aspect. Either the serum (the antibodies) or the antigen preparation is subjected to a series of measured dilutions for the purpose of determining a titer. The titer is expressed as a numerical value that signifies the highest dilution of the serum (usually) or of the antigen that gives a detectable reaction. Titers customarily are determined because a major change in titer between an acute-phase (of illness) serum specimen and a convalescent-phase specimen is diagnostic. Titers also signify levels of protection and may be indicative of the severity or the course of a disease.

Serological tests seem to have almost endless variations and applications. Over the years six kinds of antibodies have been designated: agglutinins, precipitins, antitoxins, cytolysins, opsonins, and neutralizing antibodies. Earlier serological test procedures were rather straightforward and uncomplicated when compared with today's refinements. In the earlier procedures the serum was used without fractionation or conjugation. The tests were conducted primarily in tubes and on slides. These still are basic serological tests in clinical serodiagnosis. Refinements, however, have increased the applications and the sensitivity of serological tests. The more recent techniques are of three major types:

1. Techniques for separating out the different antigens that may be present in the antigen material and techniques for separating out the different components of serum. The separations are performed in or on support media such as agar gel. Separation takes place under the influence of combinations of diffusion, osmosis, and electrical charge. Each separated antigen forms a line of precipitation with its homologous antibody.
2. Techniques for attaching soluble antigens or antibodies to cells (e.g., to red blood cells) or to particles (e.g., to latex). This allows a visible reaction (lysis or agglutination) to occur when antigen and antibody unite.
3. Techniques in which marker chemicals (labels) are conjugated to immunoglobulins (usually) or to antigens. Marker chemicals include dyes that fluoresce upon ultraviolet irradiation, radioactive materials, electron-dense materials such as ferritin that show up as uniform spheres in the electron microscope, and enzymes whose presence is detected when the appropriate substrate or chromagen or both are added to the preparation.

These basic techniques are performed in numerous ways. Gel precipitation tests, for example, may take the form of simple diffusion, double diffusion, single radial diffusion, immunoelectrophoresis, counterimmunoelectrophoresis, or rocket immunoelectrophoresis.

Monoclonal antibodies lend a high degree of specificity, uniformity, and rapidity to immunodiagnosis. Monoclonal antibodies are produced by a hybrid cell that results from the fusion of a cancerous plasma cell and an antibody-producing lymphocyte. They are being marketed commercially for a variety of clinical diagnostic procedures.

QUESTIONS FOR STUDY

1. Which two features of antigen-antibody reactions make them highly valuable for identification, diagnosis, and monitoring?
2. Describe the applications of serological test results.
3. Define *titer*. Explain how a titer is determined. What is the value of establishing a titer?
4. Name the six "classic" antibodies.
5. Explain the general principles of the more recent immunodiagnostic techniques.
6. What observable reaction occurs in agglutination? Opsonophagocytic indexing? The quellung test? The complement fixation test?
7. Explain the indicator system of the complement fixation test.
8. Name and briefly explain the methods used to separate the different molecular species of an antigen preparation or of a serum.
9. Discuss the principles of the fluorescent antibody technique. The ELISA technique. Radioimmunoassay.
10. How are monoclonal antibodies obtained?
11. In what ways are monoclonal antibody diagnostic techniques superior to conventional (i.e., polyclonal) antibody techniques?
12. Relate monoclonal antibodies to cancer immunotoxins.
13. In vivo immunodiagnostic tests lack what feature that often is part of an in vitro test?
14. To which Ig class do anti–blood group A and anti–blood group B antibodies belong?
15. Does belonging to blood group O mean that the individual has no antigens whatsoever on his or her red blood cells?
16. Since anti-Rh antibodies cross the placenta, to which Ig class must they belong?

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17. IMMUNOLOGICAL DISORDERS

OBJECTIVES

To differentiate between primary and secondary immunodeficiency diseases and to give examples of such diseases, including a description of the type of defect and the corresponding clinical manifestations

To explain the basis for categorizing hypersensitivities into the immediate or delayed types or into types I to IV

To discuss the basic differences between anaphylactic, immune complex, and cytotoxic hypersensitivity reactions; to identify the antibodies, cells, mediators, and mechanisms involved in each reaction

To discuss delayed-type hypersensitivity reactions with respect to the role of T cells, lymphokines, other influential factors, and the tissue events

To define autoimmune disease, mentioning current concepts of how it is thought to arise

To describe the role of the immune system in graft rejection and discuss maneuvers that serve to enhance the probability of graft acceptance

To comment on the role of the immune system in cancer surveillance and the potential for cancer immunotherapy

- Immunodeficiencies
  - Primary Immunodeficiencies
    - Infantile X-Linked Agammaglobulinemia
    - Di George's Syndrome
    - Severe Combined Immunodeficiency Disease
    - Immunodeficiencies in Phagocytes
  - Secondary (Acquired) Immunodeficiencies
    - Acquired Immune Deficiency Syndrome
      - Epidemiology
      - Clinical Disease
      - Possible Causal Agent and Contributing Factors
      - Effect on the Immune System
- Hypersensitivities—Allergies
  - Immediate Hypersensitivities (Humoral Hypersensitivities)
    - Anaphylaxis
      - Systemic Anaphylaxis in Humans
        - Clinical Manifestations
        - Agents of Human Anaphylaxis
        - Cellular and Tissue Events
        - The Antibody of Human Anaphylaxis
        - Management of a Systemic Anaphylactic Reaction
    - Atopy
      - Atopy and Systemic Anaphylaxis
      - Incidence and Familial Distribution
      - The Antigen of Atopic Allergies
      - The Antibody of Atopic Allergies
      - Allergy Testing
        - Types of Tests
        - False Test Reactions
      - Nonallergic Asthma and Urticaria
      - Management and Treatment of Clinical Allergies
        - Desensitization, Hyposensitization
        - Drugs, Medications, Applications
    - Arthus Reaction
      - Arthus Phenomenon in Experimental Animals
      - Underlying Mechanism
      - Arthus Reaction in Humans
    - Serum Sickness
    - Immune (Toxic) Complex Disease
      - The Diseases
      - Immune Complex Disease Factors
      - Pathogenesis of Immune Complex Disease
  - Delayed Hypersensitivity (Cellular Hypersensitivity)
    - Infectious Allergy (Tuberculin Hypersensitivity, Hypersensitivity of Infection)
      - Tuberculin Hypersensitivity
      - Delayed Hypersensitivity and the Tuberculin Reaction
      - Other Infectious Allergies
      - Diagnostic Skin Tests
      - Tuberculin Shock
      - Protective Aspects of Infectious Hypersensitivity
    - Allergic Contact Dermatitis
      - The Allergens of Allergic Contact Dermatitis
      - Allergic Contact Dermatitis Allergens as Haptens
      - Allergic Manifestations
      - Mode of Contact
      - Testing and Treatment
    - Autoimmune Disease
      - The Nature of Autoimmune Disease
      - Autoimmune Disease in Animal Model
      - Human Autoimmune Diseases
        - Myasthenia Gravis
      - Postulated Mechanisms of Autoimmune Disease
  - Graft Rejection
  - Transplantation Terminology
  - Course of Skin Allograft Rejection
  - Transplantation Antigens
  - Maneuvers to Prevent Graft Immunorejection
  - Graft-vs.-Host Disease
  - Immunologically Privileged Sites
- Tumor Immunology
  - The Imputed Role of the Immune System in Cancer Control
  - Experimental Immunotherapy of Tumors
  - Fetal Antigens and Cancer Cell Links: Active Immunization?

Disorders of the immune system stem from genetically determined developmental defects (primary immunodeficiencies), from malignancies, and from infections and drugs (secondary immunodeficiencies). Parts of the system or the entire system may fail to function, depending on the nature of the defect. There are also disorders of what is basically a normal immune system. In the hypersensitivities, the normal immune system responds to antigens in an exaggerated way that falls outside the normal response range. A number of clinical diseases result from immune disorders.

IMMUNODEFICIENCIES

Immunodefective diseases have served as "experiments of nature" that, upon analysis in the laboratory, have greatly increased the understanding of the lymphoid immune system. In turn, this understanding is leading to clinical maneuvers that are undertaken to correct immunodeficiencies. In some clinical immunodeficiency diseases it is feasible to reconstitute the individual immunologically by injecting bone marrow and lymphocytes or by transplanting thymus tissue, fetal liver, or both. The study of immunodefects has shown which component of the dual immune system is instrumental in overcoming certain infectious diseases (Table 17-1). Persons with defective B cell systems (humoral immunity) suffer a repeated succession of infections that are caused by the pyogenic cocci, by *Hemophilus influenzae*, and by *Pseudomonas aeruginosa*. These individuals cope well with fungus infections, with many of the viral infections, and with tuberculosis infections and associated reactions because their T cell system is intact. They are also able to reject foreign grafts.

PRIMARY IMMUNODEFICIENCIES

Genetically determined developmental defects of the lymphoid system may affect the B cell components (humoral immunity) only or the T cell component (cell-mediated immunity, or CMI) only. The defects may affect both the B cell and the T cell components when the developmental error is in the bone marrow stem cell population (Table 17-2). Following are brief descriptions of representative primary immunodeficiency diseases.

Infantile X-linked Agammaglobulinemia

Patients with infantile X-linked agammaglobulinemia lack B lymphocytes and therefore fail to produce immunoglobulins (agammaglobulinemia). They have a normal CMI response; that is, they are able to reject grafts and to respond to antigens that regularly elicit the CMI response. Certain areas of their lymph nodes and spleens that are responsible for antibody formation are morphologically different from those of normal organs. In the absence of antibodies the patient is highly susceptible to a succession of infections that the humoral immune component normally protects against. Immunological reconstitution is not available for this disorder. The injection of mature B cells has too many accompanying problems. Susceptibility to bacterial infection can be controlled by the injection of gamma globulin preparations as immunosupportive therapy.

Di George's Syndrome

In Di George's syndrome the thymus fails to develop; hence the failure of the thymus-dependent CMI component. The B cell population is comparatively normal. The lymphoid organs have a changed morphology. Patients with Di George's syndrome do not reject foreign grafts and do not develop delayed hypersensitivity responses. They are likely to die of infections caused by organisms such as enteric bacteria, fungi, certain viruses, and atypical mycobacteria—infections that are normally controlled by the CMI component of the immune system. Transplants of bits of wet membrane from embryonic thymus totally reconstitute T cell function. Di George patients unfortunately have an array of other defects such as hypoparathyroidism and cardiac and vascular anomalies, some of which can be lethal. Neonatal tetany caused by hypoparathyroidism may signal the immunodefect.

Table 17-1. Infection as Clue to Immune Dysfunction

Type of infection	Possible dysfunction of
Severe and/or recurrent infection with pyogenic bacteria	B cells (except in IgA deficiency) Phagocytic system (neutrophil killing defect, splenic dysfunction) Complement (deficiency of C1, C2, C3, C5, C6, C7, or C8)
Severe infection with Herpesvirus, cytomegalovirus, chickenpox, live vaccine	T cells (SCID, treated malignancy)
Hepatitis, echovirus, vaccine-strain poliomyelitis	B cells (deficiency of IgG, IgM, or IgA)
Resistant superficial candidiasis	T cells (SCID, thymic hypoplasia, chronic mucocutaneous candidiasis, steroid therapy)
Systemic infection with opportunistic fungi (e.g., *Nocardia, Aspergillus, Candida*)	T cells (Hodgkin's disease) Phagocytic system (neutrophil killing defect)
Pneumonia caused by *Pneumocystis carinii*	T cells (SCID, treated leukemia) B cells (very rare)
Giardiasis	B cells (deficiency of IgG, IgM, or IgA)
Sudden severe sepsis	B cells (deficiency of IgM) Spleen Complement

SCID = severe combined immunodeficiency disease.
Source: H. Meuwissen, Evaluating patients with suspected immunodeficiency. *Postgrad. Med.* 66:116, 1979.

Table 17-2. Manifestations of Primary Immunodeficiencies

Infection
Failure to thrive (severe T cell disease, particularly SCID)
Intestinal disorders; chronic diarrhea, malabsorption, giardiasis (common variable hypogammaglobulinemia, IgA deficiency, SCID)
Skin disorders: eczema (Wiskott-Aldrich syndrome, SCID, chronic granulomatous disease, sex-linked hypogammaglobulinemia), seborrhea (SCID), warts (IgM deficiency)
Collagen-vascular disorders: vasculitis, disease resembling systemic lupus erythematosus (deficiency of C1, C2, C4, C5, C6, C7, or C8)

SCID = severe combined immunodeficiency disease.
Source: H. Meuwissen, Evaluating patients with suspected immunodeficiency. *Postgrad. Med.* 66:116, 1979.

Severe Combined Immunodeficiency Disease

There are six genetic forms of severe combined immunodeficiency disease (SCID). In all forms, both the B and T systems are nonoperative because of bone marrow stem cell defects. Since neither protective arm of the dual immune system is functional, afflicted infants face certain death unless they are completely isolated from infectious agents or unless they are immunologically reconstituted. Successful correction has been achieved through reconstitution with bone marrow obtained from matched sibling (offspring of the same parentage) donors, with fetal liver transplants, and with a combination of fetal liver and thymus transplants.

Immunodeficiencies in Phagocytes

Chronic granulomatous disease (CGD) is a childhood disease caused by a lack of surface oxidase of the neutrophils, in consequence of which the phagocytes are unable to generate the superoxide ion (see Oxidative Killing in Chapter 14). The superoxide anion is a predecessor to the formation of hydrogen peroxide, which in turn is necessary for the formation of the bactericidal substance hypochlorite. Children with CGD cannot efficiently destroy catalase-positive bacteria such as *Staphylococcus aureus*. Another defect, similar to CGD but at a different point in the chain of chemical events that lead to oxidative intracellular killing, is myeloperoxidase deficiency. Patients apparently form normal amounts of hydrogen peroxide but lack the enzyme myeloperoxidase, which is required for the generation of hypochlorite. In Chédiak-Higashi disease, part of the defect rests in the inability of lysosomal granules to discharge their usual battery of hydrolytic enzymes.

SECONDARY (ACQUIRED) IMMUNODEFICIENCIES

Defects may arise secondarily as a result of malignancies, age, infections, and drugs that adversely affect the lymphoid system (Table 17-3). Investigations of malignancies of the lymphoid system have contributed immensely to the understanding of immunoglobulins. B cell malignancies can produce monoclonal gammopathies. *Monoclonal gammopathy* means that a B cell that multiplies uncontrollably (cancer) forms many cells of one type (clone). The cells go on to produce large amounts of one immunoglobulin class. Multiple myeloma is a malignant tumor in which one immunoglobulin class is produced in great excess.

Acquired Immune Deficiency Syndrome

A secondary immunodeficiency disease of great current interest and concern is acquired immune deficiency syndrome (AIDS).

EPIDEMIOLOGY. The Centers for Disease Control (CDC) started publishing data in 1979 about an acquired immune deficiency syndrome that especially strikes homosexual or bisexual males (73.4 percent of the cases reported through April 1985) who have contact with a large number of partners; intravenous drug abusers (17.0 percent), many of whom have a history of sexually transmitted diseases; heterosexual contact (0.8 percent); hemophiliacs (0.7 percent) who require blood clotting Factor VIII, which is prepared from the pooled plasma that has been obtained from many donors; transfusion recipients (1.4 percent); and children, who are affected while in the womb or during the perinatal period and whose mothers have AIDS or are drug abusers. Six percent of victims are female. AIDS has struck a reported 10,000 individuals through April 1985, and 4,942 (49 percent) had died. It is believed that casual contact is not involved in transmission.

CLINICAL DISEASE. Epidemiologic studies suggest that there may be a latent period from several months to 2 years between the time of exposure and clinical illness. The early stages of the syndrome have flu-like symptoms followed by a progressive illness that includes loss of weight, fever, and enlarged lymph nodes. There is an almost complete breakdown of the victim's immune system, making the person prey to opportunistic infections, cancer, and autoimmune disease. The maladies in-

Table 17-3. Some Factors Predisposing to Secondary Immunodeficiency

Factor	Principal immune component affected
Age	
Newborn (especially if premature)	B and T cells, phagocytic system, complement (severe bacterial and viral infections)
Elderly	B and T cells (anergy, autoimmunity)
Lack of breast feeding	IgA (decreased mucosal protection in intestines, increased frequency of respiratory and gastrointestinal tract infections)
Immunosuppressive therapy	
Corticosteroids	B and T cells, phagocytic system (decreased neutrophil chemotaxis)
Cyclophosphamide	B and T cells, phagocytic system (decreased number of neutrophils)
Mercaptopurine	B and T cells, phagocytic system (decreased number of neutrophils)
Irradiation	T cells, phagocytic system (decreased number of neutrophils)
Diseases involving lymphoid system	
Hodgkin's disease	T cells
Lymphoma, chronic lymphatic leukemia	B cells
Multiple myeloma	B cells (monoclonal spike on serum electrophoresis together with hypogammaglobulinemia)
Thymoma	B cells (hypogammaglobulinemia)
Splenectomy (anatomic or functional, as in sickle cell disease)	Phagocytic system (decreased clearance of pneumococci and other bacteria by spleen, macrophages)
Other	
Severe infections	T cells (decreased delayed hypersensitivity), phagocytic system (neutrophil killing defect following severe bacterial infections)
Burns	B and T cells
Malnutrition	T cells
Malignancy	T cells
Diabetes mellitus	Phagocytic system (decreased neutrophil chemotaxis)
Systemic lupus erythematosus	T cells, complement

Source: H. Meuwissen, Evaluating patients with suspected immunodeficiency. *Postgrad. Med.* 66:116, 1979.

clude Kaposi's sarcoma, autoimmune thrombocytopenic purpura (destruction of platelets by the patient's immune system accompanied by hemorrhages into the skin), and infections by a spectrum of opportunistically pathogenic organisms that include *Pneumocystis carinii, Toxoplasma gondii*, various viruses, and fungi—microorganisms that generally are controlled by CMI. Kaposi's sarcoma usually is a relatively benign cancer of the skin. There is a more aggressive and often fatal form, however, that causes internal lesions, especially of the gastrointestinal tract, cervical lymph nodes, and salivary glands. It is this virulent form that appears in about one-third of AIDS victims.

CAUSAL AGENT AND CONTRIBUTING FACTORS. The causative agent that leads to a degeneration of the immune system has recently been identified as a retrovirus that is known by several names: human T-lymphotropic virus type III (HTLV-III) or lymphadenopathy-associated virus (LAV). Factors that have been suggested to contribute to the immunodeficiency state in gays include use of amyl nitrate capsules ("poppers") or hormones that alter or

heighten sexual feelings or roles; use of purges and enemas; out-of-the-ordinary exposure to sperm, semen, and associated fluids (they are immunosuppressive in experimental animals); exposure to varied and high populations of microorganisms that barrage, blunt, or overwhelm the immune network control system.

EFFECT ON THE IMMUNE SYSTEM. The main, harmful effect on the immune system is the creation of an imbalance between helper T cells (T_H) and suppressor T (T_S) cells. There are twice as many helper cells as suppressor cells in healthy individuals. The helper-to-suppressor T cell ratio is reversed in AIDS patients. Some evidence also suggests that there is impaired lymphokine production (e.g., subnormal levels of gamma interferon), which predisposes AIDS patients to opportunistic infections. Currently, CDC defines a case of AIDS as "a disease, at least moderately predictive of a defect in cell-mediated immunity, occurring in a person with no known cause for diminished resistance to that disease."

BLOOD TESTS. Enzyme-linked immunosorbent assay (ELISA) test kits that detect antibodies to the AIDS virus became available in early 1985. The tests are used primarily to evaluate the safety of blood so that blood with AIDS antibodies does not enter the transfusion blood supply. The tests do not definitively diagnose AIDS and were not intended for that purpose.

HYPERSENSITIVITIES—ALLERGIES

The responses of the body to antigens are not always beneficial, as the term *immune* implies. The immune system may respond to an antigen in an altered way (the word *allergy* means "altered reactivity"), a way that is a departure from its normal range of responses. In most instances this is an overresponse, an excess reactivity; hence the word *hypersensitivity*. Since most allergic reactions are excessive responses, *allergy* and *hypersensitivity* have come to be used more or less interchangeably. In the hypersensitivities the concern is not with what happens to the antigen as a consequence of an antigen-antibody reaction but with what happens to the body tissues as a consequence of such a reaction.

Allergic reactions are essentially the reactions of a normal immune system. Whether an allergic response is going to occur, when it is going to occur, what type it will be, and how severe it will be are governed by a combination of factors. Among the factors are the nature of the antigen, the routes by which the antigen enters the body, the amount of antigen that enters, the chronological spacing of the exposures to the antigen, and the immune responsiveness of the patient. A number of allergic diseases are described in the following pages. Virtually all of the allergic disease types can be elicited in an experimental animal with the same antigen. It is the combination of the circumstantial factors just alluded to that determines the nature of the allergic response.

Consider what may happen when a protein antigen such as egg albumin (ovalbumin) is injected into a guinea pig. If a microgram quantity of ovalbumin is injected parenterally, and after 10–21 days a second, larger amount is injected intracardially, the animal will die within minutes of an anaphylactic shock reaction. If the ovalbumin is injected subcutaneously once a week at approximately the same site, beginning about the fourth week an inflamed area will appear that may develop into an extensive necrotic area—the typical Arthus reaction. If a single large amount of ovalbumin is injected, beginning about the sixth day the animal will have enlarged joints, one of the manifestations of serum sickness. If the ovalbumin is given with Freund's adjuvant, a subsequent challenge with ovalbumin may lead to the formation of tubercle-like lesions in the lung, a reaction that is typical of tuberculin hypersensitivity. If a parenteral injection is given every several days, the animal will produce a high-titered precipitating serum. If the antigen in the latter instance is an immunogen such as a toxoid, the production of the high-titered serum will be interpreted as a beneficial, protective response. This merits repetition: Most allergic diseases are determined by a combination of factors.

Table 17-4. Principal Characteristics of Immediate and Delayed Hypersensitivities

	Type of allergy	Examples of antigens	Coupler: Ig class or T cells	Principal effector cells and their mediators	Principal pathophysiological effects
IMMEDIATE Humoral hypersensitivity Antibody-mediated Passively transferable via serum	Systemic anaphylaxis	Penicillin Hymenoptera stings Foreign serum	IgE	Mast cells: Histamine SRS-A Kinins	Capillary permeability; smooth muscle contraction; exocrine secretions
	Atopic allergies (localized anaphylaxis)	Pollens House dust Animal epidermals	IgE	Mast cells: Histamine SRS-A Kinins	Exocrine secretions
	Arthus reaction	Ovalbumin Foreign serum	IgG and/or IgM plus complement	Cells of acute inflammation	Induration and dermonecrosis owing to localized destruction of blood vessels
	Serum sickness	Foreign serum Depot penicillin	IgG and/or IgM plus complement; some IgE	Cells of acute inflammation; mast cells	Vasoactivity, edema, inflammation; hives, arthralgia, lymph node enlargement
	Immune (toxic) complex	Soluble antigens Viruses	IgG or IgM plus complement	Cells of acute inflammation	Kidney (basement membrane) damage; damage to other organs and tissues; vasculitis
DELAYED	Tuberculin (infectious allergy)	Tuberculin Fungi	T cells	Lymphocytes: Lymphokines Macrophages	Antigen lysis or isolation Destruction of host cells and tissues that surround antigen

Table 17-4 (continued)

	Type of allergy	Examples of antigens	Coupler: Ig class or T cells	Principal effector cells and their mediators	Principal pathophysiological effects
DELAYED, CONT'D Cell-mediated hypersensitivity Sensitized lymphocyte–mediated Passively transferable via lymphocytes and transfer factor	Allergic contact dermatitis	Poison ivy Industrial chemicals Cosmetics			
	Graft rejection	Foreign tissues, organs, cells			
	Cancer surveillance	Cancer cells			
	Autoimmune disease	"Self" antigens	T cells and antibodies	Lymphokines and immune complexes	

SRS-A = slowing-reacting substance of anaphylaxis.

A similar variety of allergic responses in humans is experienced with penicillin. Penicillin injected parenterally into a sensitized person occasionally leads to death because of anaphylactic shock reaction. Penicillin applied topically may lead to allergic contact dermatitis. If administered in a form that persists in the tissues, penicillin may lead to a serum sickness–like reaction.

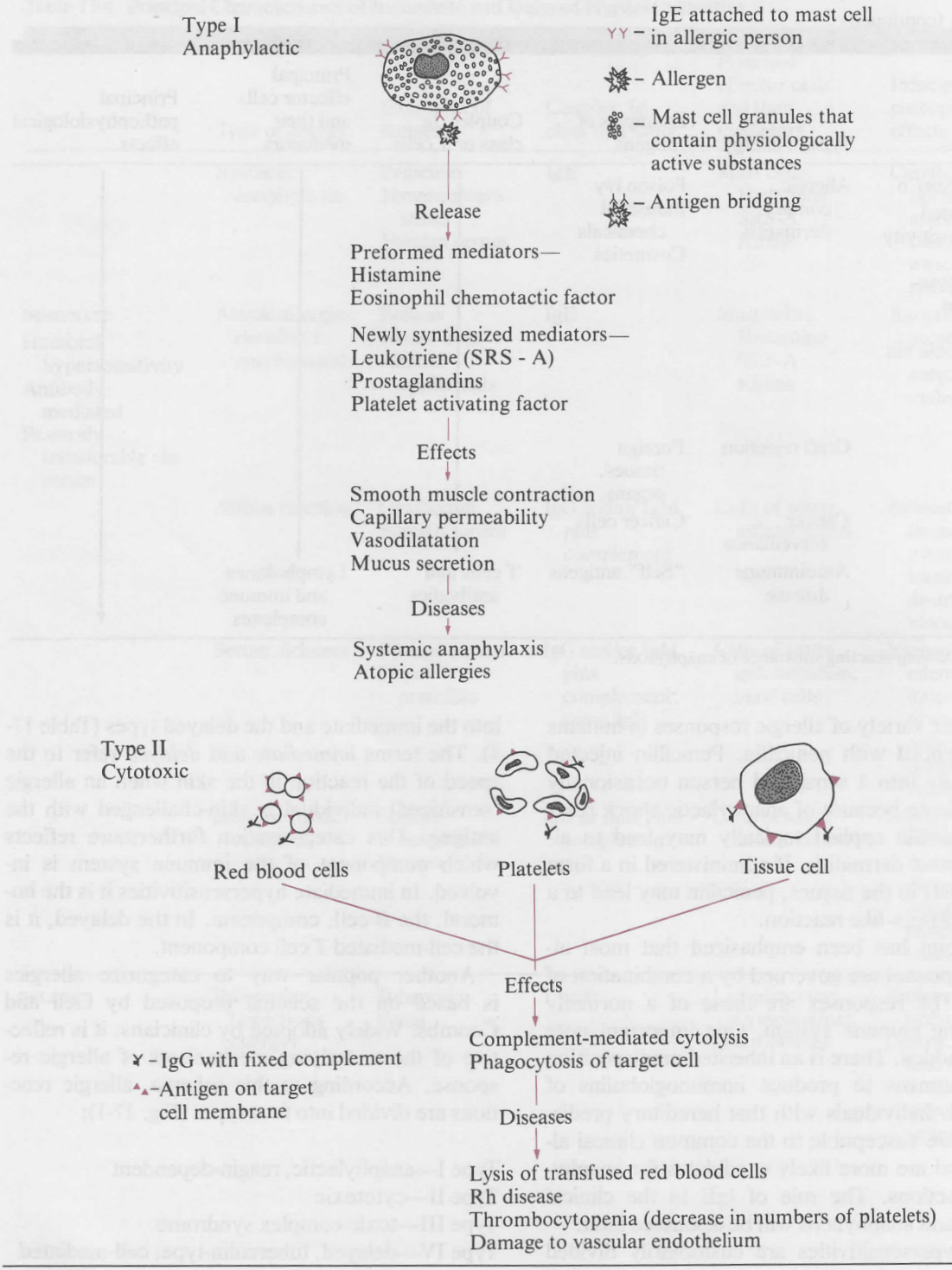
The point has been emphasized that most allergic responses are governed by a combination of factors. The responses are those of a normally functioning immune system. One important note must be added. There is an inherited predisposition among humans to produce immunoglobulins of class IgE. Individuals with that hereditary predisposition are susceptible to the common clinical allergies and are more likely candidates for anaphylactic reactions. The role of IgE in the clinical allergies and anaphylaxis will be discussed later.

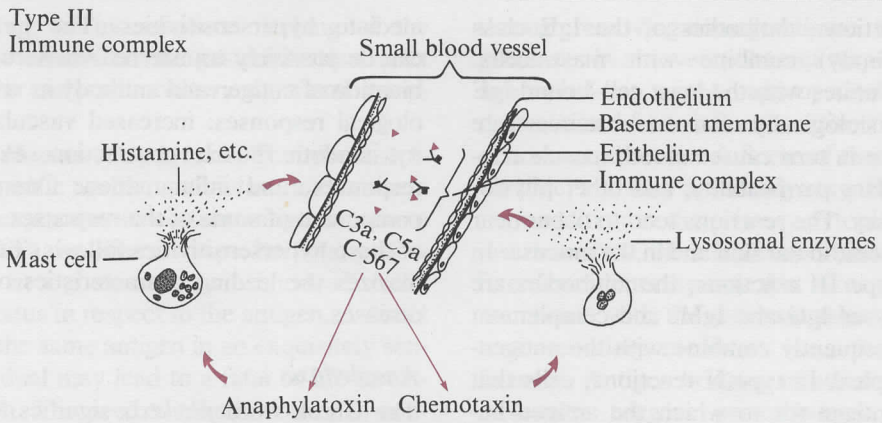
The hypersensitivities are customarily divided into the immediate and the delayed types (Table 17-4). The terms *immediate* and *delayed* refer to the speed of the reaction in the skin when an allergic (sensitized) individual is skin-challenged with the antigen. This categorization furthermore reflects which component of the immune system is involved. In immediate hypersensitivities it is the humoral, the *B* cell, component. In the delayed, it is the cell-mediated *T* cell component.

Another popular way to categorize allergies is based on the schema proposed by Gell and Coombs. Widely adopted by clinicians, it is reflective of the underlying mechanisms of allergic response. According to this schema, allergic reactions are divided into four types (Fig. 17-1);

Type I—anaphylactic, reagin-dependent
Type II—cytotoxic
Type III—toxic-complex syndrome
Type IV—delayed, tuberculin-type, cell-mediated

Fig. 17-1. Types of hypersensitivity. (SRS-A = slow-reacting substance of anaphylaxis; T_{DTH} cell = delayed-type hypersensitivity T cell.)





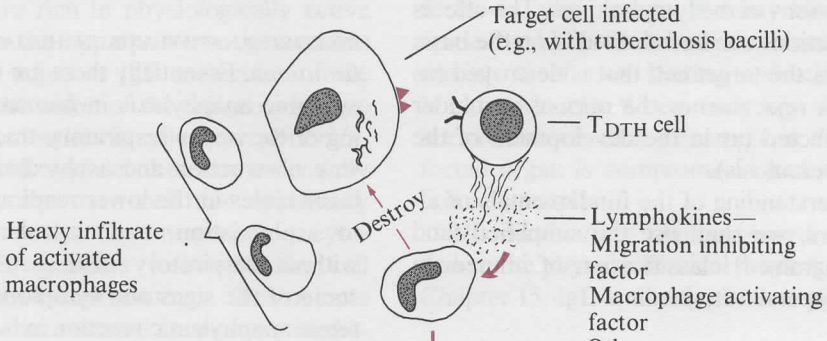
Effects

Vasculitis
Destruction of basement membrane

Diseases

Arthus reaction
Serum sickness
Autoimmune disease

Type IV
Delayed (cell-mediated)



Effect

Destruction of target cell
and/or surrounding tissue cells

Diseases

Tuberculosis, fungus infections
Graft rejection
Allergic contact dermatitis

In type I reactions, antibodies of the IgE class (reaginic antibody) combine with mast cells. When antigen unites with the mast cell–bound IgE antibody, physiologically active substances are released. These in turn cause smooth muscle contraction, capillary permeability, and other physiological responses. The reactions tend to occur near the body surfaces, in the skin and in the mucosa. In type II and type III reactions, the antibodies are usually of classes IgG and IgM, and complement components frequently combine with the antigen-antibody complex. In type II reactions, cells that contain the antigen (or to which the antigen becomes attached) are the target cells of the reaction. The attachment of the antibody to the antigen-bearing cell causes the cell to be destroyed. In type III reactions, the effect of the antigen-antibody union is realized not on the antigen but on normal cells and tissues of the body. They are referred to as *innocent bystander cells*. The inflammatory events triggered by the antigen-antibody combination bring injury to these normal bystander cells. In type IV reactions, it is the cell-mediated arm of the immune system that causes the immune disorder through the actions of the lymphokines. The effects of type IV reactions can be subdivided on the basis of whether it is the target cell that is destroyed (as occurs in graft rejection) or the normal bystander cell that is affected (as in the development of the tubercle in tuberculosis).

For an understanding of the fundamentals of allergic disorders, we shall use the simplified (and historically ingrained) classification of immediate and delayed hypersensitivities.

IMMEDIATE HYPERSENSITIVITIES (HUMORAL HYPERSENSITIVITIES)

In the immediate hypersensitivities the sensitized individual reacts within minutes when challenged with the antigen that induced the allergic state. With the skin test the response is in the form of a wheal-and-erythema reaction (see Fig. 17-2) (described under Atopy). Antibodies, as opposed to the lymphocytes of CMI, are involved in the immediate hypersensitivities. The hypersensitivity can be passively transferred via serum. The combination of antigen and antibody in vivo sets off biological responses: increased vascular permeability, smooth muscle contraction, exocrine gland responses, and inflammation. Complement is a component of some of the responses. A discussion of these hypersensitivities follows. Table 17-4 summarizes the leading characteristics of the allergic diseases.

Anaphylaxis

The Greek word *phylaxis* signifies "guarding" or "protection." Combined with the negative *ana-*, the word *anaphylaxis* is translated to mean "unguarded" or the reverse of protecting. Individuals can be "unguarded" in respect to an antigen. They are "sensitized" to the antigen. If in this anaphylactic, unguarded condition they encounter the same antigen under certain circumstances, an anaphylactic reaction develops within minutes. The response may be so extreme that the person undergoes a life-threatening systemic reaction, anaphylactic shock, which in some instances results in death.

SYSTEMIC ANAPHYLAXIS IN HUMANS. *Clinical Manifestations*. Essentially there are three syndromes of systemic anaphylaxis in humans: extensive swelling of the upper respiratory tract that leads to airway obstruction and asphyxiation, spasms of the bronchioles in the lower respiratory tract that lead to asphyxiation, and vascular collapse (shock) without respiratory distress. The following are some of the signs and symptoms present in a systemic anaphylactic reaction in humans: reactions of the skin in the form of a diffuse erythema (reddening), or raised, blanched areas known as *urticaria* (hives), and pruritus (intense itching); hypotension (i.e., shock resulting from increased vascular permeability and collapse); obstruction of the upper respiratory tract, especially as the result of laryngeal edema; pulmonary involvement, usually manifested as an asthma-like syndrome but sometimes owing to fluid (edema); and smooth muscle spasms, evidenced particularly in the genitourinary and gastrointestinal tracts as abdominal pain, diar-

rhea, vomiting, or incontinence. Itching of the scalp and the effects on the gastrointestinal tract are among the early premonitory signs and symptoms.

Agents of Human Anaphylaxis. The administration of therapeutic agents, antibiotics, and antisera and natural exposures to certain antigens (venom of Hymenoptera, i.e., bees, wasps, hornets, yellow jackets) may at a given unknown time lead to the unguarded status in respect to the antigen. A direct exposure to the same antigen in an exquisitely sensitized individual may lead to a fatal or life-threatening reaction. The need to take histories, to conduct appropriate tests, to heed warnings from previous reactions, and to know the conditions that are likely to produce such reactions should be apparent to those who are responsible for giving injections to patients. Six illustrative cases of fatal systemic anaphylaxis in humans are summarized in Table 17-5.

Cellular and Tissue Events. The sensitizing dose or doses of soluble antigen evoke the production of antibodies of the IgE class (see Fig. 17-1, type I). IgE antibodies attach to host mast cells and basophils, cells that are rich in physiologically active substances. When the appropriate antigen makes contact with two adjacent IgE molecules of the same specificity (the union of the antigen with two neighboring IgE antibodies is called *antigen bridging*) on the mast cell surface, there are essentially two responses by the mast cell that apparently proceed simultaneously. One response is cytoplasmic—mast cell granules fuse with the cell membrane and release their contents. The contents of the granules that are released are primary mediators such as histamine, chemotactic factors such as eosinophil chemotactic factor and phagocyte chemotactic factor, and proteins, many of which are of the neutral protease class. Histamine's principal effector functions are the altering of vascular permeability and the contraction of smooth muscle. Chemotactic factors attract various cell types to the site of foreign substance intrusion. The importance of the neutral proteases is not yet understood, but it is reasonable to conjecture that they are involved in early tissue repair processes through removing damaged tissue. Conversely, the proteases in an overexuberant response, as occurs in anaphylaxis, might inappropriately engage in the destruction of normal connective tissue. The mediators are short-lived. Some of their effects are reversible, and they are slowly resynthesized.

In addition to the primary cytoplasmic response just described, there is a concomitant and probably interdependent response that takes place in the cell membrane with the resultant release of highly potent secondary mediators (i.e., they are not preformed). Antigen bridging of adjacent specific IgE molecules triggers perturbations (sequences of changes) of the mast cell's phospholipid membrane. Secondary mediators that are lipid mediators are formed through the oxidative metabolism of arachidonic acid. The secondary mediators, which are not derived from the secretory granules, are prostaglandins, thromboxanes, and leukotrienes. The leukotrienes (LTA4 to LTE4) were for many years called SRS-A—the slow-reacting substance of anaphylaxis. They are potent chemotactic, vasoactive, and spasmogenic agents.

When there is a great abundance of these effects—in other words, if many blood vessels become "leaky"—fluid from the blood vessels pours out into the tissues, leading to shock and blockages in blood vessels. When many key smooth muscles are contracted, as for example in the lung, the affected organ is compromised and ceases to function adequately.

The Antibody of Human Anaphylaxis. In addition to the physicochemical properties described in Chapter 15, IgE antibody has these features:

1. In certain contexts IgE is called *reagin, skin-sensitizing antibody*, or *anaphylactic antibody*. This same immunoglobulin class is involved in atopic allergies (localized anaphylactic reactions).
2. IgE antibody has a predilection to become attached by the Fc (fragment, crystallizable) end of the antibody molecule to mast cells and basophils. For this reason it is said to be *cytotropic*.
3. Some IgE antibody is present in the serum and is therefore passively transferable via serum.

Table 17-5. Clinical Data from Six Cases of Human Anaphylaxis

Case no.	Sex	Age (yr)	Agent	Dose (ml)	Route of administration	Estimated time from challenge to death (min)	Symptoms and signs	Known prior exposure	"Allergic history"
1	F	39	Penicillin	1.5	Intramuscular	60	Generalized warmth; tightness of throat; respiratory distress; cyanosis; convulsion; respiratory failure	Yes	"Hives" 2 weeks before death; allergen unknown
2	F	21	Guinea-pig hemoglobin	0.2	Subcutaneous	16	Headache; wheezing; cyanosis	No	"Asthma"; skin sensitivity to dog hair, kapok, shellfish, ragweed, timothy, orris root, and house dust
3	M	52	Bee venom	—	Subcutaneous	20	Unknown	Yes	Severe local reaction to bee sting 20 years previously
4	M	45	Penicillin	—	Intramuscular	60	Dyspnea	Unknown	Unknown
5	M	56	Hay-fever desensitization vaccine	1/16	Subcutaneous	45	Difficulty breathing	Yes	"Hay fever"—injection was 11th in series of weekly desensitization injections
6	F	38	Penicillin and streptomycin	—	Intramuscular	120	Chest pain; cough; collapse; hypotension; cardiac arrest	Unknown	

Source: L. P. James and K. F. Austen, Fatal systemic anaphylaxis in man. *N. Engl. J. Med.* 270:598, 1964.

Table 17-6. Common Clinical Allergies

Allergic respiratory disease (ARD)
- Pollinosis—seasonal allergic rhinitis. Pollens are from trees and grasses in spring and early summer, weeds in late summer. Pollens are mostly wind-borne but also insect-borne.
- Perennial allergic rhinitis (PAR)—year-round. Common allergens are house dust and animal epidermals (hair, feathers, dander). Among the many allergens of house dust are mold spores, animal epidermals, and mites.
- Bronchial asthma. Common allergens are those cited under ARD and PAR. The air sacs are overdistended, plugs of mucus fill bronchial passages, smooth muscles enlarge (hypertrophy) to thicken and narrow the walls of the bronchi. Symptomatic relief is obtained by bronchodilators that relax the muscles of the bronchi, and by expectorants and liquefacients that dissolve and expel the mucous plugs and the other accumulations.

Gastrointestinal allergies—colic and possibly ulcerative colitis

Allergic skin disorders
- Atopic dermatitis. This can be divided into stages: infant, childhood, and adolescent-adult.
- Allergic contact dermatitis (ACD). A variety of allergens (contactants), many serving as haptens, elicit nonatopic skin allergies via the delayed hypersensitivity mechanism.

A beneficial function of IgE in normal individuals can only be surmised. Exocrine glands respond to the mediators to provide a flushing of the mucous surfaces. This may be useful to combat infections of the gastrointestinal tract, especially parasitic worm infections. The vasoactive and smooth muscle responses on a lesser, localized level may serve the purpose of retarding an irritant or of removing it from the bloodstream to localize it in the tissues.

Management of a Systemic Anaphylactic Reaction. Increased permeability of blood vessels and smooth muscle contraction are the principal effects of the mediators of an anaphylactic reaction. Top priority is accorded to epinephrine as a countermeasure to control an ongoing systemic anaphylactic reaction. Epinephrine is a physiological antagonist of histamine; that is, it counteracts the effects of histamine. It dilates bronchioles, has a vasoconstricting influence, and aids in the resorption of edema fluid. It also blocks the release of mediators of allergy. If shock is severe, drugs must be administered to maintain blood pressure. Upper airway obstruction owing to laryngeal edema may necessitate some form of airway maintenance.

Atopy

In 1923 Coca applied the term *atopy* to the common clinical allergies, recognizing that there was a hereditary predispostion of family members to develop allergies. The atopic hypersensitivities are of the immediate type. They are naturally or spontaneously occurring and are due to the everyday inhalation of, ingestion of, or contact with the antigen. The atopic hypersensitivities are largely those associated by the layperson with the term *allergy*: asthma, hay fever, hives (urticaria), and food allergies. Most of the atopic allergies are clinical allergies—those for which the patient goes to see the physican for diagnosis, treatment, and control (Table 17-6).

ATOPY AND SYSTEMIC ANAPHYLAXIS. The atopic allergies are also called *reaginic* allergies, *clinical* allergies, the *common* allergies, or *local anaphylaxis*. When IgE was discovered, it was recognized that the mechanism of systemic anaphylaxis and of the atopic allergies was the same from the standpoint of the antibody type involved, the general mechanism of release of physiologically active substances as a result of antigen-antibody combination, and the pharmacological effects of those mediators. The atopic allergies (localized anaphylaxis) differ from systemic anaphylaxis in degree. Systemic anaphylaxis affects the smooth muscles and blood vessels throughout the body and in a more violent, sudden manner. The antigen in systemic anaphylaxis generally enters the body by a more direct route—by injection (e.g., penicillin shots) and by penetration through the skin as in insect stings.

Table 17-7. Allergens and Other Stimuli Provoking Bronchopulmonary Disease

Provoking stimuli in bronchial asthma	Allergens of importance in bronchial asthma	Organic dusts of hypersensitivity pneumonitis
Immunological reactions	Inhalants	Mold growths in
Immediate hypersensitivity (type I)	Tree, grass, weed pollens	Hay
Immune complex (type III)	Mold spores	Redwood dust
Infection	Animal danders, feathers	Maple bark dust
Viral (in upper or lower respiratory tract)	House dust (mites, cotton linters, etc.)	Oak bark dust
Acute bacterial sinusitis or bronchitis	Pyrethrum (insecticide spray)	Mushroom dust
Chronic sinusitis	Orris root (cosmetics)	Wheat flour weevil
Aspirin intolerance	Insect parts (moths, cockroaches, etc.)	Contaminated humidifiers and air conditioners
Isoproterenol abuse	Ingestants	Vegetable dusts
Inhalation of irritant smoke, fumes	Foods (grains, nuts, eggs, etc.)	Sisal
Exercise	Drugs (penicillin)	Coffee
Atmospheric change	Injectants	Enzyme detergents
Emotional upset	Hymenoptera stings (bee, wasp, hornet, yellow jacket)	Pituitary snuff
Associated disease states	Drugs (penicillin, allergy extracts, adrenocorticotropic hormone)	
Chronic obstructive pulmonary disease		
Cardiac failure		
Esophageal disorder		

Source: D. A. Mathison and D. D. Stevenson, Bronchopulmonary diseases, immunologic perspectives. *Postgrad. Med.* 54:105, 1973.

INCIDENCE AND FAMILIAL DISTRIBUTION. It is estimated that 10 percent of the population have minor allergies that are detected when they are appropriately tested for. A history of allergy among close relatives can be obtained among a majority of allergic patients. The allergic syndrome in the offspring is not necessarily the same as that of the parents. The allergic substance may well be different, and the clinical type may be different. Indeed, the target organ within the same individual may change. The first allergic disease experienced in infancy may be a food allergy with gastrointestinal involvement, progressing through allergic dermatitis in childhood, and eventually developing into asthma in young adulthood.

THE ANTIGEN OF ATOPIC ALLERGIES. The routes of sensitization are primarily through the respiratory mucosa, the gastrointestinal tract mucosa, and the skin; hence the allergic substances are spoken of as *inhalants, ingestants, contactants*, or *injectants* (Table 17-7, center column). The antigen or allergic substance is usually called an *allergen*. The word *allergen* is most frequently used in relation to the atopic (local anaphylaxis) allergies. It can, however, also signify the antigen in the other hypersensitivities.

THE ANTIBODY OF ATOPIC ALLERGIES. The antibody of atopic allergies has a number of names: *reagin* (to be distinguished from the antibody in syphilis, also called *reagin*), *reaginic antibody, skin-sensitizing antibody, P-K antibody* (see Prausnitz-Küstner test in the next section), *atopic antibody, anaphylactic antibody*, and *IgE*. It is the same immunoglobulin class, IgE, that is involved in systemic anaphylaxis.

ALLERGY TESTING. The tests described here are primarily tests for atopic allergies, but several apply

as well to other allergic types and other clinical conditions. Many of the antigen preparations for allergic testing are commercially available, but some may have to be tailored by the allergist to the patient's history or idiosyncrasies.

Types of Tests

1. Skin tests. The introduction of the allergen to which the patient is allergic leads within minutes (immediate) to the wheal-and-erythema reaction, which is also referred to as the triple-response reaction (Fig. 17-2). The site first reddens (erythema); as edema fluid collects in the area, the central portion becomes raised and blanched while the periphery becomes erythematous. Skin testing can be in the form of a scratch test. The surface of the skin is abraded with a dull needle or implement, and a droplet of allergen is instilled. Skin testing may be intradermal, measured amounts of allergen being injected between the layers of the skin. The intradermal test is the more sensitive of the two skin tests. It carries the hazard of a summation of reactions if the patient is allergic to a number of tested antigens. Many allergens can be tested at the same time by positioning the injection sites in checkerboard fashion on the skin of the patient's back or forearm or on the lateral aspect of the upper arm.

2. Conjunctival instillation. A dilution of a suspected allergen is introduced into the conjunctival sac of the eye. Redness, lacrimation (tearing), and itching are signs and symptoms of a positive reaction. Sensitivity to foreign sera is customarily tested in this manner.

3. Patch test. The suspected allergen is either directly taped to the skin or applied to gauze or cotton that is then taped to the skin. This method is used with some of the delayed allergies, especially with the contact dermatitides.

4. Prausnitz-Küstner (P-K) test. The P-K test is a passive transfer test. It is not always possible, or it may be inadvisable because of an exquisite sensitivity, to test the allergic individual directly. Instead, blood is obtained from the patient and serum is collected. A small amount of the serum is injected into the skin of a volunteer. Skin-sensitizing antibody (IgE), if present, is passively transferred into the skin of the volunteer. Several hours later—time for the IgE to attach—the suspected allergen is injected into the site. The typical wheal-and-erythema response develops in a positive test.

Fig. 17-2. Whealing reactions following intradermal testing. (From B. F. Feingold, *Introduction to Clinical Allergy*, 1973. Courtesy of Charles C Thomas, Publisher, Springfield, Illinois.)

False Test Reactions. Allergy testing is not without problems. There are false-positives and false-negatives. Appropriate antigens are not always available. Testing for food allergies deserves the added comment that the allergenic component of the food is in many cases a derived antigen. The natural food is altered in the gastrointestinal tract by the digestive juices to give rise to different antigens from those that were present in the preingested food.

NONALLERGIC ASTHMA AND URTICARIA. The symptom complex that is seen in asthma and urticaria (hives) is not always allergic in origin (see Table 17-7, left column). The mediators of the atopic allergies can also be discharged in response to emotions (neurogenic mechanism), heat, cold, and other triggering agents.

MANAGEMENT AND TREATMENT OF CLINICAL ALLERGIES. The order of preference is avoidance of the allergen, reduction of the patient's sensitivity by desensitization or hyposensitization, and relief of the symptoms with drugs, medications, and applications.

Desensitization, Hyposensitization. It is possible to lessen (hyposensitize) or eliminate (desensitize) certain allergic states by parenteral injections of the corresponding allergen. Best success is attained in hay fever. The parenterally introduced antigen ("allergy shots" to the patient) elicits the formation of IgG antibodies. They are termed *blocking antibodies* because they are believed to interfere with the action of the reagins. The IgG antibodies are believed to have a greater affinity for the allergen, thereby forestalling the stimulation of more homologous IgE antibodies and preventing the allergen from interacting with the IgE antibodies. An alternative explanation is that the desensitization that results from the administration of the allergen is due to the increased production of suppressor T cells.

Drugs, Medications, Applications. Drugs used to relieve or manage common allergic reactions and states fall into the general categories of antihistamines, decongestants, bronchodilators, vasoconstrictors, expectorants, and corticosteroids. Antihistamines, of little value in systemic anaphylaxis because of the suddenness and extent of the reaction, are mainstays in allergic rhintis and urticaria.

Arthus Reaction

ARTHUS PHENOMENON IN EXPERIMENTAL ANIMALS. The subcutaneous injection of a highly antigenic soluble substance leads to vasculitis (inflammation of blood vessels) and tissue necrosis if the injection is repeated at intervals of about one week. The first several injections produce no visible response. Beginning with about the third or fourth injection at approximately the same site in the skin, an ever-increasing area of tissue damage develops (Fig. 17-3). The lesion changes from a small reddened spot to an edematous inflammation that progresses to extensive areas of necrosis and sloughing of tissue. Blood vessel damage is the principal factor in the pathogenesis.

Fig. 17-3. Two Arthus reactions in rabbit skin. Larger reaction has extensive zone of erythema and edema surrounding its necrotic center. (From J. T. Barrett, *Textbook of Immunology* [4th ed.]. St. Louis: Mosby, 1978.)

UNDERLYING MECHANISM. When a slight excess of soluble antigen combines with antibody in vivo, the immune complex that is formed binds complement factors (see Fig. 17-1, type III). That combination leads to the damaging inflammation of the blood vessels. Small thrombi (clots) are formed in the vessels, neutrophils are attracted to the site, and basophils and mast cells discharge vasoactive substances into the site. If the reaction is severe enough, blood vessels and the tissues they service are destroyed.

ARTHUS REACTION IN HUMANS. The Arthus reaction is regarded as a localized reaction in the tissues. In humans it occurs in some unusual forms of local insulin allergy, from repeated injections of tetanus toxoid, and from the inhalation of a variety of antigens. Inhalation of antigens leads to the condition known as *extrinsic allergic alveolitis*, which is also referred to as *hypersensitivity pneumonitis*. Inhaled antigens include plant dusts that are contaminated with bacteria or fungi (farmer's lung), animal proteins (pigeon breeder's lung), and various microorganisms that are aerosolized by forced-air systems (air-conditioner or humidifier lung) (see Table 17-7, right column). The Advisory Committee on Immunization Practices recommends that the new 23-valent polysaccharide vaccine against *Streptococcus pneumoniae* be given only once because Arthus reactions and systemic reactions have been common in adults receiving second doses.

Serum Sickness

The administration of a large amount of foreign serum leads to a systemic condition known as *serum sickness*. In 6–12 days after the injection of the serum the patient typically experiences arthralgia (painful, swollen joints), fever, urticaria, and lymphadenopathy (enlargement of lymph nodes and spleen). A distinguishing feature of serum sickness reactions is that only one dose of antigen is necessary, a large or persistent dose. One dose serves as both the antibody-eliciting dose and the reaction-provoking dose. The body usually clears foreign proteins uneventfully. A large or a persisting quantity of antigenic material, however, requires a longer time for elimination. Antibody formation starts promptly when the serum is injected. Beginning at about the sixth day, antibody is present at a level sufficient to react with remaining antigen to create the serum sickness. Once the antigen has been eliminated, the serum sickness subsides.

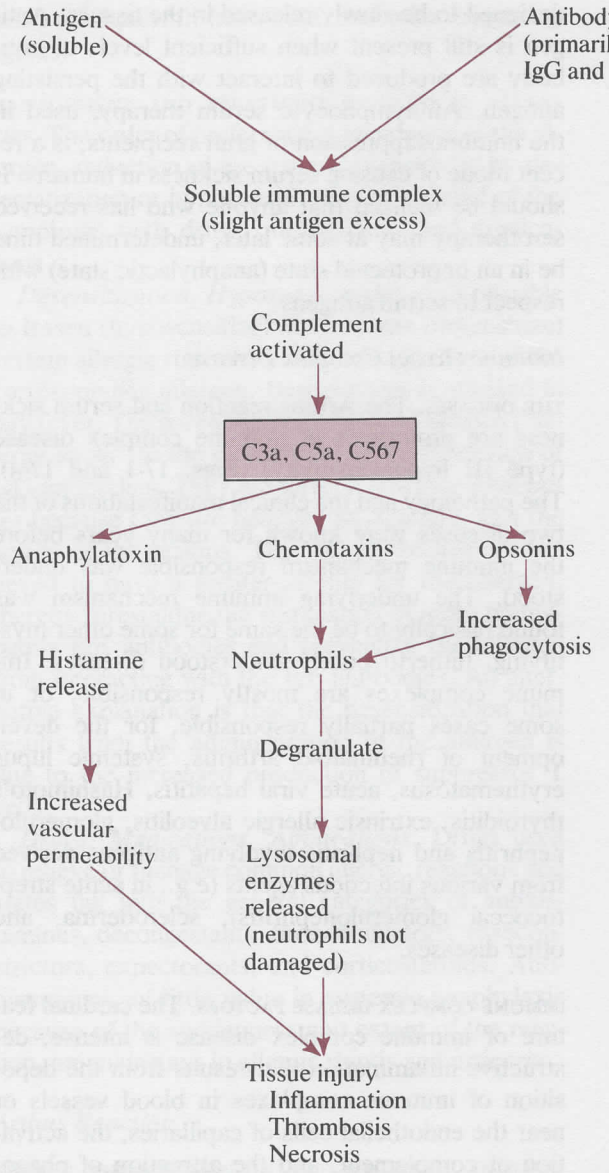
Serum sickness was encountered more frequently before the advent of the antibiotics when serotherapy was more widely used in infectious disease management. The antigen need not be serum. A large, persisting amount of antigen leads to the clinical syndrome. Currently serum sickness is clinically important with certain chemotherapeutic agents—for example, with antibiotics designed to have a prolonged action. Benzathine penicillin is designed to be slowly released in the tissues. Antigen is still present when sufficient levels of antibody are produced to interact with the persisting antigen. Antilymphocyte serum therapy, used in the immunosuppression of graft recipients, is a recent mode of causing serum sickness in humans. It should be realized that anyone who has received serotherapy may at some later, undetermined time be in an unprotected state (anaphylactic state) with respect to serum antigens.

Immune (Toxic) Complex Disease

THE DISEASES. The Arthus reaction and serum sickness are prototypes of immune complex disease (type III hypersensitivity) (Figs. 17-1 and 17-4). The pathology and the clinical manifestations of the two diseases were known for many years before the immune mechanism responsible was understood. The underlying immune mechanism was found basically to be the same for some other mystifying, hitherto poorly understood diseases. Immune complexes are mostly responsible, or in some cases partially responsible, for the development of rheumatoid arthritis, systemic lupus erythematosus, acute viral hepatitis, Hashimoto's thyroiditis, extrinsic allergic alveolitis, glomerulonephritis and nephritis involving antigens derived from various infectious agents (e.g., in acute streptococcal glomerulonephritis), scleroderma, and other diseases.

IMMUNE COMPLEX DISEASE FACTORS. The cardinal feature of immune complex disease is intense, destructive inflammation that results from the deposition of immune complexes in blood vessels or near the endothelial cells of capillaries, the activation of complement, and the attraction of phagocytes to the involved area. The antigens are usually soluble: proteins, nucleic acids, viruses, bacterial components, and others. The antibodies are of class IgG or IgM, the only immunoglobulin classes

Fig. 17-4. Immune complex disease. The antigen-antibody complexes (microprecipitates) are formed in the blood and in tissue spaces and deposited in the walls of blood vessels, in basement membranes, and in joint synovia.



Examples of antigens
Exogenous
- Serum, toxoids, drugs
- Bacteria
- Viruses
- Parasites
- Fungi
- Bird proteins

Endogenous
- Nuclear antigens
- Immunoglobulins
- Thyroglobulin
- Tumor antigens

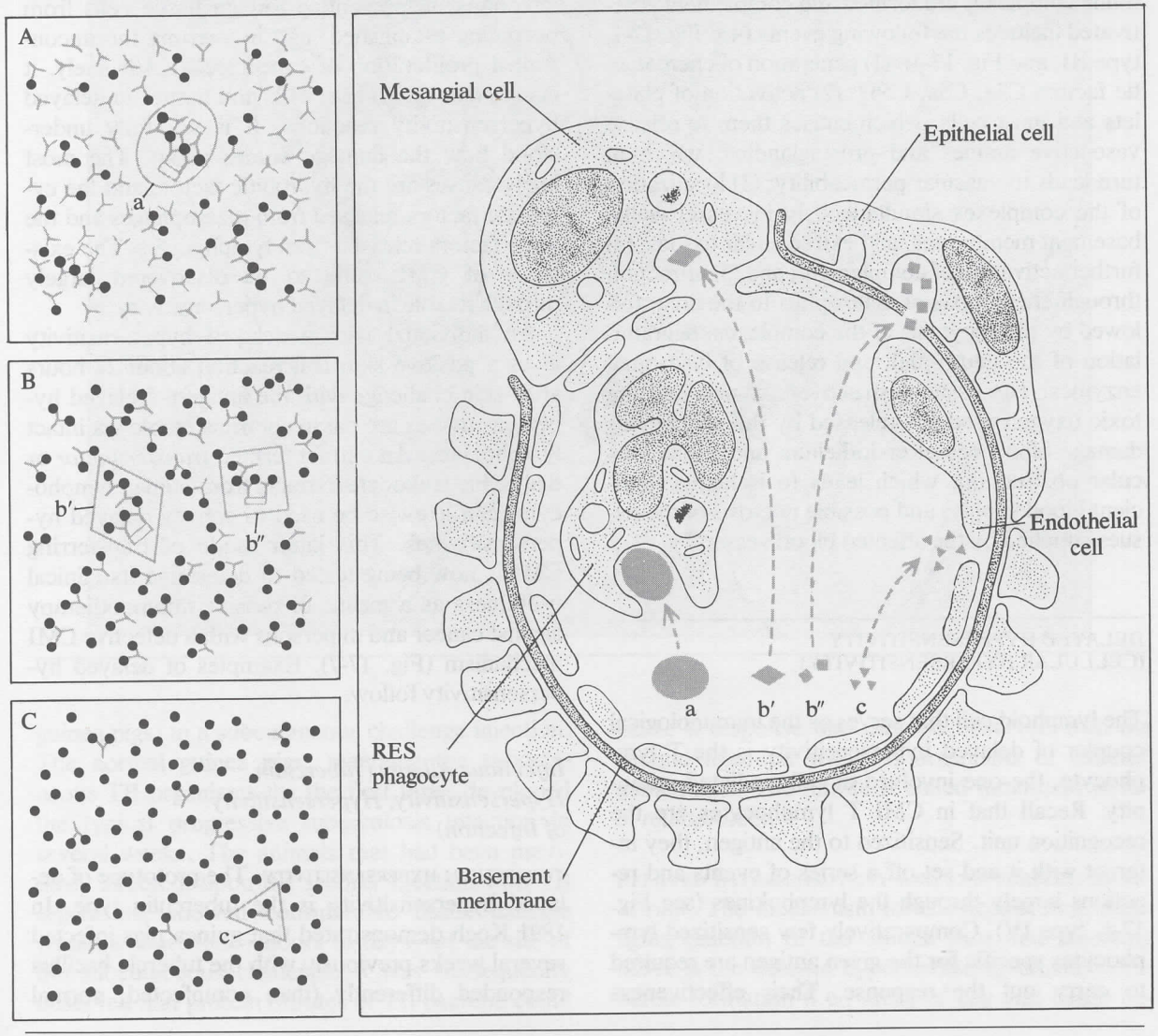
Examples of immune complex involvements in disease
Glomerulonephritis
- Poststreptococcal glomerulonephritis

Pneumonitis
- Farmer's lung (moldy hay)
- Pigeon breeder's lung (bird proteins)

Vasculitis
- Arthus reaction

Synovitis
Lymophadenitis
Fever
Serum sickness

Fig. 17-5. Relative concentrations of antigen and antibody in initiating immune complex–associated disease. A key factor in determining whether immune complexes initiate kidney diseases is the relative concentrations of antigen and antibody in the serum. The small panels at left depict the effects of this relationship. A. At one extreme, a large antibody excess results in an extensive lattice and very large complexes that come out of solution or are phagocytized before reaching the kidney. C. At the opposite extreme, if there is a large antigen excess, the size of the complexes is held below that necessary for fixing complement. Such complexes are not inflammatory. B. Between those two extremes, when there is a modest antigen excess, the complexes are large enough to fix complement but not so large as to be intercepted by reticuloendothelial system (*RES*) phagocytes. Within this kidney-damaging range, as shown in the large panel at right, the size of the complexes is a determinant of where they will become localized. The larger complexes (*b′*) are most likely to be taken up by the phagocytic mesangial cells; the smaller ones (*b″*) are most likely to pass through the glomerular or tubular basement membranes and be trapped by the foot processes of the epithelial cells. (Figure by B. Tagawa. From C. B. Wilson and F. J. Dixon, *Hosp. Pract.* 14[4]:57, 1979. Reproduced with permission.)



that regularly bind complement. The antigen-antibody quantitative relationship usually is such that the antigen is in slight excess of antibody or that the relationship is close to equivalence (equal proportions) (Fig. 17-5). When there is a great excess of either antigen or antibody the complexes are rapidly cleared by the reticuloendothelial system before damaging inflammatory responses are initiated.

PATHOGENESIS OF IMMUNE COMPLEX DISEASE. The pathogenesis of immune complex disease after immune complexes are formed and complement is activated includes the following events (see Fig. 17-1, type III, and Fig. 17-4): (1) generation of chemotactic factors C3a, C5a, C567; (2) activation of platelets and mast cells, which causes them to release vasoactive amines and prostaglandins, which in turn leads to vascular permeability; (3) localization of the complexes simultaneously in vessel walls, basement membranes, and extracellular tissues; (4) further activation of complement; and (5) attraction through chemotaxis of neutrophils to the area, followed by phagocytosis of the complexes, degranulation of the neutrophil, and release of lysosomal enzymes. The tissue-destructive enzymes and the toxic oxygen products released by the phagocytes damage blood vessel endothelium and cause vascular obstruction, which leads to ischemia (deficient blood supply) and possible necrosis of the tissues supplied by the affected blood vessels.

DELAYED HYPERSENSITIVITY (CELLULAR HYPERSENSITIVITY)

The lymphoid cell that serves as the immunological coupler of delayed hypersensitivity is the T lymphocyte, the one involved in cell-mediated immunity. Recall that in CMI T lymphocytes are the recognition unit. Sensitized to the antigen, they interact with it and set off a series of events and reactions largely through the lymphokines (see Fig. 17-1, type IV). Comparatively few sensitized lymphocytes specific for the given antigen are required to carry out the response. Their effectiveness against the offending antigen is amplified in a number of ways. They themselves, upon antigenic contact, proliferate. What is more important is that they attract macrophages and keep them from migrating from the scene of conflict (Fig. 17-6). The macrophages, once activated by the lymphokines, deal with the antigen by phagocytosis, through hydrolytic enzymes, and with other destructive properties. The lymphocyte itself (T_C) can be toxic for host cells that express foreign antigens.

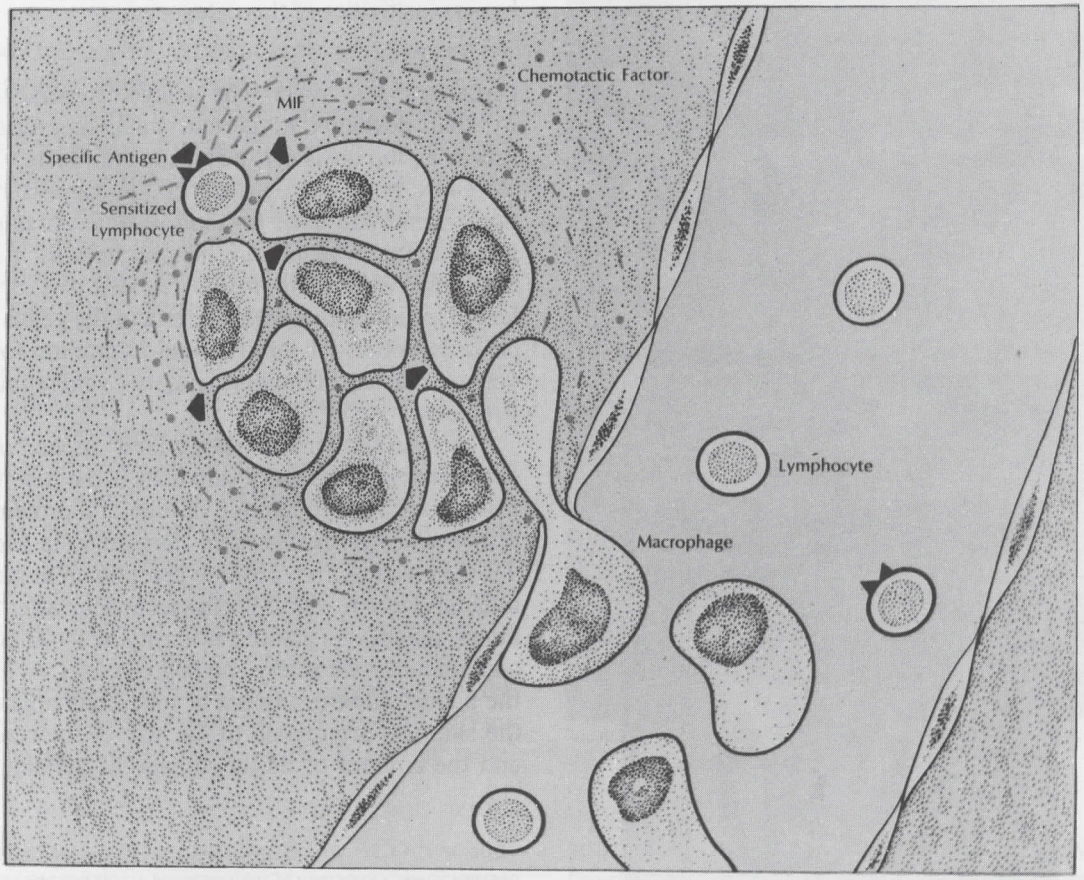
The CMI mechanism, as has been previously noted, is beneficial in overcoming certain types of infections, in preventing foreign tissue cells from becoming established, and in averting the uncontrolled proliferation of cancer cells. Adversely, it causes damage to host cells and tissues in delayed hypersensitivity reactions. It is not fully understood how the damage comes about. The most likely causes are the hydrolytic factors and the cytotoxic factors released from macrophages and the toxic factors released from lymphocytes. The existence of CMI came to be discovered largely through its role in delayed hypersensitivity.

An individual with a delayed hypersensitivity gives a positive skin test reaction about 24 hours after skin challenge with the antigen. Delayed hypersensitivities are passively transferable via intact lymphocytes. An extract termed *transfer factor* or dialysable leukocyte extracts from human lymphocytes can likewise be used to convey delayed hypersensitivities. This latter mode of transferring CMI is now being tested to determine its clinical usefulness as a means of passive immunotherapy against cancer and in persons with a defective CMI mechanism (Fig. 17-7). Examples of delayed hypersensitivity follow.

Infectious Allergy (Tuberculin Hypersensitivity, Hypersensitivity of Infection)

TUBERCULIN HYPERSENSITIVITY. The prototype of delayed hypersensitivity is the tuberculin type. In 1891 Koch demonstrated that guinea pigs infected several weeks previously with the tubercle bacillus responded differently (than noninfected, normal

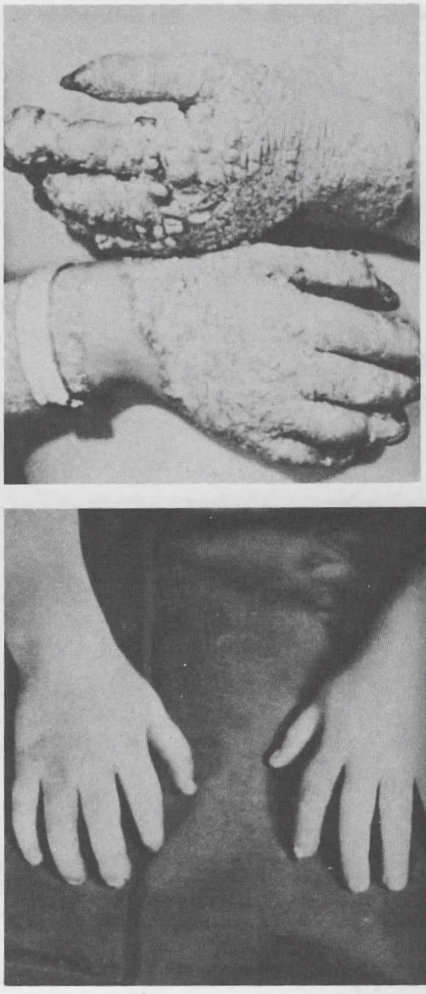
Fig. 17-6. Role of chemotactic factor and migration inhibiting factor (*MIF*) in the cellular hypersensitivity reaction. If an animal is challenged with an antigen to which it has been sensitized, a small number of specifically sensitized lymphocytes make contact with antigen at the challenge site, and chemotactic factor and MIF are elaborated. Chemotactic factor attracts macrophages to the area. MIF keeps them there and activates them. (Figure by I. Geis. From J. R. David, *Hosp. Pract.* 6[3]:79, 1971.)



guinea pigs) to a subcutaneous challenge injection. The normal guinea pigs, receiving the subcutaneous TB organisms for the first time, developed the typical progressive tuberculosis infection in several weeks. The animals that had been previously infected, upon the second injection with TB organisms, showed considerable tissue damage within a short time—hours to days—at the site of the subcutaneous challenge. It was subsequently observed that protein filtrates of TB bacteria could cause a response like the one observed with the second injection with TB organisms of infected guinea pigs. The filtrate is called *tuberculin* or *tuberculoprotein*.

DELAYED HYPERSENSITIVITY AND THE TUBERCULIN REACTION. The mechanism for the accelerated, damaging reaction of the animal skin and adjoining tissue is the delayed hypersensitivity reaction. Tubercle formation (i.e., areas of cell and tissue ne-

Fig. 17-7. Therapeutic use of transfer factor: hands of child with mucocutaneous candidiasis before and after transfer factor was administered. (From H. H. Fudenberg, et al., The therapeutic uses of transfer factor. *Hosp. Pract.* 9[1]:95, 1974.)



crosis), as occurs in tuberculosis, is largely due to the delayed hypersensitivity mechanism. The host becomes allergic to tuberculoprotein, and the lymphocytes sensitized to that protein cause tissue damage at the infected site through the cell-mediated mechanism.

OTHER INFECTIOUS ALLERGIES. Other infectious agents, particularly organisms that cause chronic infections or agents that are difficult to eliminate by other defensive and immune processes, produce delayed hypersensitivities. Fungi especially and certain parasites, bacteria, and viruses give rise to hypersensitivities of this category.

DIAGNOSTIC SKIN TESTS. The delayed hypersensitivity that develops in certain infectious diseases serves as the basis for diagnostic skin tests. Commercially prepared extracts of the various infectious agents are used for skin testing. Among the preparations available are histoplasmin for fungus infections with *Histoplasma capsulatum*, Brucellergen for infections with bacteria of the genus *Brucella*, lepromin for leprosy, and Lygranum for the venereal disease lymphogranuloma venereum (the Frei test).

The best known and most widely applied of the delayed hypersensitivity skin tests is the tuberculin test. A purified preparation of tuberculoprotein, referred to as *purified protein derivative*, is used in TB skin testing. In a positive reaction an indurated inflammatory reaction begins to appear after 6–12 hours, reaches its greatest intensity in 24–72 hours, and gradually subsides. The degree of sensitivity of the patient and the amount of tuberculin applied in the skin test are related to the size of the reaction and the amount of tissue damage in the skin test site.

PROTECTIVE ASPECTS OF INFECTIOUS HYPERSENSITIVITY. The response of the immune system to the TB organisms and other organisms that elicit a delayed hypersensitivity response should not be regarded as entirely deleterious. Initially the Koch phenomenon described previously was considered a defensive, beneficial response. Certain of its aspects, combined with humoral immune response, are protective. It is not possible at this time to provide a clear-cut protection against the diseases in humans without concomitantly producing the undesirable features of delayed hypersensitivity.

Allergic Contact Dermatitis

Allergic contact dermatitis (ACD) ordinarily results from exposure to simple chemical allergens via the skin. Clinical allergists categorize ACD as a "nonatopic" allergy to distinguish it from the immediate, hereditarily predisposed atopic clinical allergies.

THE ALLERGENS OF ALLERGIC CONTACT DERMATITIS. The most common causes of ACD are simple chemicals: soaps, cosmetics, industrial chemicals, ointments, chemotherapeutic agents (especially those that are topically applied), and plant substances, to mention a few. The classic example of ACD involves plants in the genus *Rhus*: poison ivy, poison oak, and poison sumac. The active allergenic factor is an oleoresin.

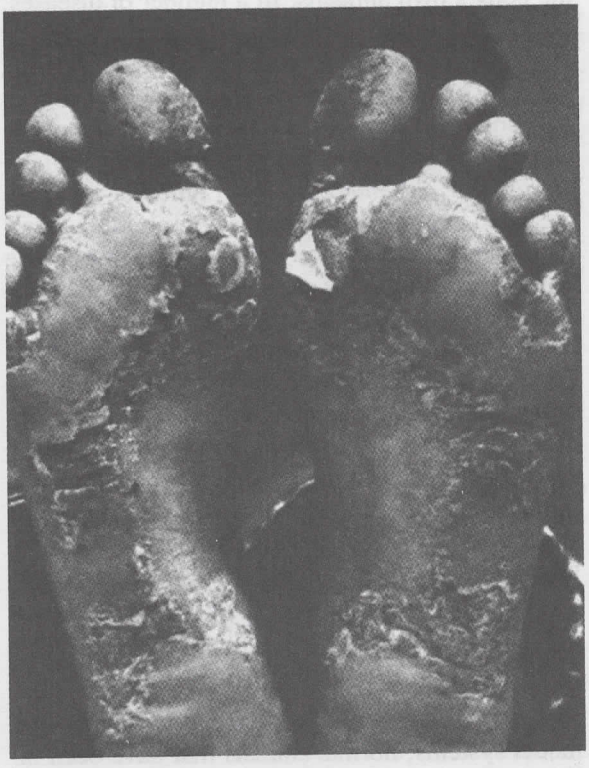
ALLERGIC CONTACT DERMATITIS ALLERGENS AS HAPTENS. ACD allergens are often substances of relatively simple chemical composition. The point was emphasized in an earlier chapter that antigens are usually large molecules, with a molecular weight of at least 10,000. How then can simple chemicals such as nickel, mercury, and the catechols of *Rhus* plants be allergenic? The commonly accepted theory is that they act as partial antigens, that is, as haptens. They conjugate with proteins of the skin. The complete antigen consists of the haptens of the allergenic substances as the determinant groups and the proteins of the skin as the carrier molecules.

ALLERGIC MANIFESTATIONS. An acute ACD reaction, occurring on the average 18–24 hours after a sensitized individual encounters the allergen, may range from a well-defined erythematous area to extensive areas of vesiculation (bullae). The vesiculated (blistered) lesions may ooze, crust, and become infected (Fig. 17-8). Pruritus (intense itching) is almost always present. The oleoresin of the *Rhus* plants may be spread early by rubbing or contact before the vesicular reaction occurs.

MODE OF CONTACT. Usually there is a history of repeated direct contact with the allergen. But a sensitized person experiencing a reaction may not have a history of direct contact. With poison ivy, for example, pets and clothing may be the indirect bearers of the stimulus. Burning of the plants may vaporize the allergenic components, and the smoke, coming in contact with exposed parts of the body, may give rise to a violent exacerbation.

TESTING AND TREATMENT. The customary mode of testing for hypersensitivity is the patch test method as described under Atopy. Treatment is usually in the conservative form of compresses and soaks. Steroids are used in severe cases.

Fig. 17-8. Pruritic scaly eruption caused by delayed hypersensitivity reaction to adhesive in lining of tennis shoes. (From S. M. Connolly, *Postgrad. Med.* 74:227, 1983. By permission of Mayo Foundation.)



Autoimmune Disease

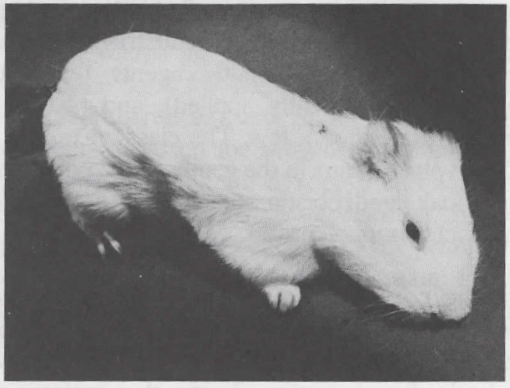
THE NATURE OF AUTOIMMUNE DISEASE. A cardinal tenet of immunology is that "self" antigens be recognized so that an immune attack is not made on the body's own antigens. In a number of diseases, however, it appears that the self-recognition fails or that body proteins are somehow altered, allowing the body's own cells, molecules, tissues, or organs to become the target for attack by the immune system. Such diseases are referred to as *autoimmune diseases* or *autoallergies*. Both humoral and cell-mediated immune mechanisms contribute to them, with immune complexes frequently involved. The immune system in its pathogenetic role may not necessarily be the sole cause of the disease. Perhaps the proteins, cells, and tissues that become involved in what is labeled as an autoimmune disease are undetectably diseased or defective. The immune system recognizes the abnormality of these components and institutes measures to rid the body of them or to alter them. Autoantibodies have been demonstrated against gamma globulins (the rheumatoid factor in rheumatoid arthritis); against nuclei (in disseminated lupus erythematosus); against red blood cells, platelets, and granulocytes; and against various tissue components.

AUTOIMMUNE DISEASES IN ANIMAL MODELS. In animal model systems, normal animals that have been injected with various antigens, such as homogenates of normal brain tissue, lens protein, thyroglobulins, or testicular materials, have developed diseases resembling human diseases: allergic encephalomyelitis, thyroiditis, aspermatogenesis, and myasthenia gravis (Fig. 17-9).

HUMAN AUTOIMMUNE DISEASES. A number of human diseases are regarded as having autoimmune disease components: Hashimoto's chronic thyroiditis, systemic lupus erythematosus, rheumatoid arthritis, Sjögren's syndrome, scleroderma, autoimmune hemolytic anemia, and myasthenia gravis.

Myasthenia Gravis. Acquired myasthenia gravis will be described because the cause, the probable pathogenetic mechanisms, and the management of the patient have been extensively investigated.

Fig. 17-9. Experimental autoimmune myasthenia gravis (EAMG) in guinea pig. The low head with chin and elbows on the floor, hunched back, abducted thighs, and flexed digits form a posture characteristic of EAMG in the guinea pig. (From V. A. Lennon, J. M. Lindstrom, and M. E. Seybold, Experimental autoimmune myasthenia: A model of myasthenia gravis in rats and guinea pigs. *J. Exp. Med.* 141:1365, 1975.)



Myasthenia gravis victims experience weakness of striated muscles. The severity of the disease depends on which muscles are involved and the extent of the involvement. If respiratory and bulbar muscles (i.e., muscles controlled by the medulla oblongata) are extensively affected, the condition, if untreated, is life-threatening.

The cause of myasthenia gravis is known insofar that it involves an autoantibody to the acetylcholine receptor proteins on the muscle end-plate. Muscle contraction normally occurs when acetylcholine released from the neuron binds to its receptor protein on the muscle end-plate.

Several mechanisms have been proposed as to how the autoantibody affects the normal acetylcholine-receptor interaction: The autoantibody attaches to the actual binding site of the receptor molecule (only part of the receptor molecule serves as the binding site), thereby blocking acetylcholine's access to the site; the union of the autoantibody to a nonbinding site of the receptor molecule activates complement (see Immune Complex Disease) and this complex triggers the destruction of the muscle

cell membrane; a single autoantibody molecule cross-links two receptor molecules and thereby accelerates the breakdown phase of the normal breakdown-regeneration rate of receptor molecules.

Treatment, which is tailored to the patient's condition, may include the use of drugs that inhibit acetylcholinesterase so that the catabolism of acetylcholine is decreased, leaving enough acetylcholine to produce an adequate signal; removal of the autoantibodies by plasmapheresis; immunosuppressive drugs; thymectomy; and the administration of oral corticosteroids.

POSTULATED MECHANISMS OF AUTOIMMUNE DISEASE. Certain conditions are believed to be instrumental in initiating autoimmune disease:

1. Antigens may be sequestered. Certain tissues of the body never become marked as self in fetal or neonatal life. They are anatomically separated or develop later. As a result of injury, surgery, or some other agency the antigens from these tissues may be exposed to the immune system. Antibodies or sensitized lymphocytes are then formed. They attack the originally sequestered tissue to cause, for example, thyroiditis, aspermatogenesis, and uveitis (inflammation of the middle coat of the eye).
2. Normal body proteins may be altered to an antigenic form by drugs, chemicals, or infectious agents. Rheumatic fever results from such an alteration through an infection with *Streptococcus pyogenes*.
3. Normal body proteins may cross-react with antibodies that were produced against foreign antigens that are antigenically related to body proteins. Certain antigens of a bacterial cell may so closely resemble proteins of the body that the antibodies generated against the former will interact with the latter as well in a self-destructive manner.
4. The immune recognition system may be defective. It may not have developed fully or in the normal time span, or there may be an imbalance between helper T cells and suppressor T cells.

GRAFT REJECTION

The immunology of transplantation is usually discussed under delayed hypersensitivity because the principal mechanism of tissue immunorejections is delayed hypersensitivity. It is a cherished dream of humanity to be able to routinely replace defective diseased tissues and organs with normal tissues and organs. Immunorejection is regarded therefore as a disservice by the immune system. In actuality, immunorejection under normal circumstances is a beneficial reaction; without it the body could serve as a culture medium for any cell or tissue it contacts.

TRANSPLANTATION TERMINOLOGY

Certain prefixes (*auto-, iso-, allo-, xeno-*) and certain suffixes (*-graft, -antigen, -antibody, -geneic*) are used to form terms that are part of transplantation parlance (Table 17-8). Some of the words so constructed are applicable in other areas of immunology as well. Autografts and isografts are not immunorejected. Most grafts between humans are allografts (formerly called *homografts*). The donor and recipient are allogeneic; that is, they are members of the same species but they are not genetically alike. The tissues of the donor are foreign to the recipient and are therefore antigenic. Xenografts are so genetically disparate that the immunologically normal individual totally rejects them. Animal tissues and organs are therefore not likely sources of replacements for diseased human organs.

COURSE OF SKIN ALLOGRAFT REJECTION

A skin allograft shows clinical signs of rejection in 10–14 days in what is described as a *first-set rejection*. Sensitized lymphocytes and reactive lymph nodes can be detected beginning about the sixth day. Later, mononuclear cells infiltrate and damage the vascular bed of the graft. The graft becomes

Table 17-8. Transplantation Terminology

Prefix	Meaning	Combining suffixes	Transplantation parlance
Auto-	Self	-graft -geneic -antigen -antibody	An autograft is a self graft, e.g., skin from one site of the patient's body moved to another site on the patient's body
Iso-	Equal; identical with another	-graft -geneic -antigen -antibody	An isograft is a graft between isogeneic individuals, i.e., between genetically identical individuals such as identical (uniovular) twins
Allo- (homo-)	Similar; like another	-graft -geneic -antigen -antibody	An allograft is a graft between allogeneic individuals, i.e., between nonidentical members of the same species
Xeno- (hetero-)	Dissimilar; unlike another; foreign	-graft -geneic -antigen -antibody	A xenograft is a graft between xenogeneics, i.e., between members of different species, as a graft from ape to human

necrotic and is sloughed. If a second skin allograft from the same donor is transplanted to the sensitized recipient, graft rejection is accelerated. The *second-set rejection* takes place in 5–6 days, so rapidly that vascularization may not occur. In the second-set rejection the recipient already has antibodies and sensitized cells. The second graft is analogous to a booster dose that stimulates an already primed system.

TRANSPLANTATION ANTIGENS

The antigens of the graft are called *transplantation antigens*. They are controlled by histocompatibility genes of the major histocompatibility complex as described in Chapter 15. The key to successful grafting is to select the most appropriate donor by matching the donor and the recipient at the major histocompatibility complex (called HLA in humans). The greater the genetic disparity between the donor and the graft recipient, the greater the likelihood of an immunorejection reaction.

MANEUVERS TO PREVENT GRAFT IMMUNOREJECTION

The approaches used to forestall immunorejection include the following:

1. Tissue typing. The purpose of tissue typing is to match the tissue antigens of the donor and recipient as closely as practical conditions allow. The HLA (human leukocyte antigens) are located on a small segment of human chromosome 6. The products of some of the gene loci on this segment can be serologically identified. In kidney grafting long-term survival of the graft occurs in a high percentage of cases when there is a sibling donor who is serologically matched with the recipient at both the HLA-A and HLA-B loci and when appropriate immunosuppression accompanies the grafting. Once the donor has been selected on the basis of the HLA matching, the donor's lymphocytes are mixed with the recipient's serum to detect cytolytic antibodies. Transplantation is not feasible if there are cytolytic antibodies. ABO blood group status is also determined, not for the purpose of tissue typ-

ing but for compatibility in case transfusion between donor and recipient is required.

2. Immunosuppression. The purpose of immunosuppressive procedures is to prevent the recipient from forming antibodies and sensitized lymphocytes, to prevent lymphocyte proliferation, and to destroy those that are already formed. There are three main immunosuppressive modalities: (a) Roentgenographic radiation has the objective of destroying the x-ray–sensitive antibody-forming lymphoid tissue. Irradiation has been largely replaced by the other immunosuppressive modalities. (b) Immunosuppressive drugs include antimetabolites (e.g., azathioprine), which prevent the proliferation of mitotically active cells. Lymphoid tissue, responsible for antibody formation and for the lymphocytes of CMI reactions, is mitotically active. Anti-inflammatory drugs, especially the glucocorticoid prednisone, are used in synergistic combinations with the antimetabolites for their lymphocyte-destroying (lympholytic) actions. (c) Antilymphocyte serum is a powerful immunosuppressive agent. Antibodies to human lymphocytes are produced in horses. The horse serum is processed to obtain the antibody-containing globulin fraction (the globulin fraction in this instance is called *antilymphocyte globulin*, a treatment that is designed to eliminate immunologically the T lymphocytes, the principal couplers of delayed hypersensitivity reactions and therefore graft rejection. The immunosuppressed patient has little or lowered resistance to infectious agents including many opportunistically pathogenic microorganisms such as gram-negative bacteria, fungi (*Candida*), the protozoan *Pneumocystis carinii*, and cytomegalovirus. Pneumonia is a constant threat. Tumor formation in graft recipients also occurs at significantly elevated rates when there has been effective immunosuppression. Discontinuance of immunosuppression often causes tumors that have been unknowingly transplanted with the donor tissue to languish or to disappear.

GRAFT-VS.-HOST DISEASE

An interesting reaction, graft-vs.-host disease (GVHD), can occur when the recipient of a graft has a defective immune system or response. The defect may be congenitally determined or result from immunosuppression. If an allograft contains sufficient immunocompetent cells (lymphocytes), those cells can attack the recipient's cells and tissues. In experimental animals immunocompetent grafts have been able to cause runting and death of the recipient. The graft literally rejects the recipient. GVHD was a major deterrent in the early efforts to immunologically reconstitute immunodefective individuals with stem cell (bone marrow, fetal thymus, fetal liver) transplants. The advent of HLA matching techniques has made reconstitution much more successful in about twenty otherwise fatal immunodeficiency diseases. GVH reaction, incidentally, does not result from an early fetal liver transplant.

IMMUNOLOGICALLY PRIVILEGED SITES

Allografts to certain sites are not ordinarily immunorejected. For example, the avascularity of corneas apparently prevents the foreign antigens of the graft from reaching the immune response system, and so tissue typing and immunosuppression are not required in corneal transplantation. Poorly vascularized areas of the brain and testes are other such immunologically privileged sites.

TUMOR IMMUNOLOGY

THE IMPUTED ROLE OF THE IMMUNE SYSTEM IN CANCER CONTROL

The increased incidence of tumor formation in the immunosuppressed and the decline of transplanted tumors after the discontinuance of immunosuppression seem to signify that the immune system plays an important role in preventing cancer.

The transplant donors in most instances were not known to have had tumors so either their immune system kept the tumors in check or else the tumors were derived from the transplant recipient once his or her immune system was suppressed. Cancers furthermore tend to occur early in life when the immune system is not fully developed or in later years when the immune system is on the decline. Children with congenital defects, especially of the cellular immune system, experience a high incidence of tumors of the lymphatic system.

EXPERIMENTAL IMMUNOTHERAPY OF TUMORS

It is now generally accepted that tumor-specific antigens exist—that tumor cells contain antigens different from the normal antigens of body cells. That there are tumor antigens and that the immune system, especially the CMI system, seems to be charged with destroying abnormal cells offer the possibility of employing immunotherapy as an additional weapon in the armamentarium against cancer. The possibilities include the administration of lymphokines, sensitized cells (cytotoxic lymphocytes), immunotoxins (see discussion of monoclonal antibodies, Chapter 16), and natural killer cells; controlling suppression; and immunization with fetal antigens (next paragraph).

FETAL ANTIGENS AND CANCER CELL LINKS: ACTIVE IMMUNIZATION?

Fetal antigens in animals and fetal-antigen substitutes in humans are being investigated for immunization against tumors. Experimentally induced and spontaneous tumors in animals and spontaneous tumors in humans sometimes produce fetal antigens. Serological findings show that fetal antigens appear in certain human tumors: carcinoembryonic antigen in intestinal and gastric tumors and in fetal gut; alpha-fetoprotein in liver tumors of humans and of mice; blood group precursor substances in tumors of gastric and intestinal origin. These discoveries imply that fetal genes are "switched back on" in the transformation to cancer and fetal antigens become reexpressed in malignant cells. The reason that fetal antigens are being investigated for immunization is that they appear to incite antiembryo immunity and that immunity seems to afford protection against tumors. For example, female mice of high parity (having borne many young) are more resistant to induced tumors than are nulliparous (no births) animals. Tumors of the human female reproductive organs reportedly are more common in childless than in parous (childbearing) women, and the incidence of tumors declines as parity increases.

Antiembryo immunity incidentally is different from the maternal immune response to the fetus. The fetus is like a genetically foreign object, like a graft, to the mother's immune system because any noncompatible paternally determined antigens of the fetus are foreign to the mother's immune system.

SUMMARY

The immune system does not always provide protection, even though that is its primary function. The immune response may be deficient because of developmental or acquired defects, or the response may be outside the normal range, as occurs in allergies. Developmental defects determined by heredity are referred to as primary immunodeficiencies. They are usually manifested in neonates and children. Acquired defects result from malignancy, infections, drugs, and aging and are referred to as secondary immunodeficiencies.

Hypersensitivities usually develop because of a combination of factors such as the amount and type of antigen (allergen) and the route and spacing of exposure. Hereditary predisposition is a factor in IgE-mediated allergies. Hypersensitivities are categorized as immediate or delayed, depending on how soon a sensitized individual reacts when skin-tested with the appropriate antigen. The immediate

type are antibody mediated, the delayed type are cell mediated. There are four types of hypersensitivities based on the mechanism of allergic injury. Type I are the IgE-mediated, hereditarily predisposed local (atopic) and systemic anaphylactic type. IgE antibodies that are attached to mast cells from prior sensitization, when bridged by more of the same antigen, activate mast cells to release pharmacologically active substances. The substances affect blood vessels, smooth muscles, and exocrine glands. The effects of these responses range from localized allergic manifestations (atopic allergies) to the systemic and sometimes fatal reactions of anaphylactic shock. In type II hypersensitivities host cells are damaged or destroyed when antigens that are part of the host cell surface or that become attached to host cells react with antibody. Red blood cells and platelets often are involved. In type III hypersensitivities, the immune complex type, host tissues are damaged because of the vascular effects and inflammatory reactions that are initiated by complexes formed by soluble antigen, antibody of classes IgG or IgM, and complement. In type IV hypersensitivities, the delayed or CMI type, tissue damage results from the activation of cytotoxic T cells or is mediated by lymphokines.

Immunopathologists frequently include descriptions of graft rejection, cancer surveillance by the immune system, and the immunology of the fetal-maternal relationship with type IV hypersensitivities because of the involvement of CMI in these clinical entities.

QUESTIONS FOR STUDY

1. How do primary immunodeficiencies differ from secondary immunodeficiencies?
2. Briefly describe infantile X-linked agammaglobulinemia, Di George's syndrome, and severe combined immunodeficiency disease, and tell how knowledge of the basis of these defects helped in understanding the B and T cell systems of immunity.
3. What is a monoclonal gammopathy, and how does it relate to monoclonal antibodies?
4. How are the following related to AIDS?

   Opportunistic pathogens
   Kaposi's sarcoma
   Pneumocystis carinii
   Human T–lymphotropic virus type III
   Helper T and suppressor T cells
5. What is the clinical basis for categorizing hypersensitivities as immediate or delayed, and how do the two types differ?
6. Describe the immune mechanisms and the immunopathological differences among types I, II, III, and IV hypersensitivities.
7. What are the signs and symptoms of human systemic anaphylaxis?
8. Discuss the tests that are used in clinical allergy diagnosis.
9. What are the immunological conditions under which immune complex disease occurs?
10. How does a positive tuberculin test differ from a positive atopic allergy skin test?
11. How are many of the allergens of allergic contact dermatitis related to haptens?
12. Describe the major modes of preventing allograft immunorejection.
13. Why are fetal antigens being investigated for active immunization against tumors?
14. Define, describe, or explain

HLA system	Blocking antibody
Atopy	Hyposensitization
P-K test	Graft-vs.-host disease
Antigen bridging	Allograft
Urticaria	Sibling
Leukotriene	Ingestant

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V. BACTERIA THAT CAUSE INFECTIOUS DISEASE

18. INTRODUCTION TO THE BACTERIAL DISEASES

OBJECTIVES

To summarize how bacterial diseases and the bacteria that cause them are explained in a taxonomically organized medical microbiology textbook

To apply the information about the regularly cited properties of bacteria

To relate the meanings of the listed prefixes and suffixes to terms that are frequently used in medical microbiology

Chapter Topics
- General Characteristics
- Diseases Caused by Members of the Family or Genus of Bacteria
  - Signs and Symptoms
  - Pathogenesis and Pathology
  - Bacterial Virulence Factors and Other Important Factors
- Laboratory Identification
  - Overview of the Identification Process
  - Importance of Identifying Bacterial Strains
  - Identification of Nonpathogens
  - Laboratory Identification Procedures
    - Microscopical Characteristics
    - Cultural Characteristics
    - Biochemical Characteristics
    - Serological Characteristics
    - Animal Identification
- Epidemiology
- Treatment
  - Chemotherapy
  - Passive Immunotherapy
  - Other Forms of Treatment
- Prevention and Control
  - Active Immunization
  - Prophylactic Passive Immunotherapy
  - Sterilization and Disinfection Measures

Study Aids
- Regularly Cited Bacterial Properties
- Organizational Aid
- Terminology
- Prefixes
- Suffixes

It is necessary to introduce a few concepts that should make it easier to interpret the vast amount of information about bacterial disease agents. Many of the statements and principles that have been applied to bacterial agents of disease will also apply to other agents of disease, namely viruses, fungi, and animal parasites.

CHAPTER TOPICS

This textbook uses a taxonomic approach to the discussion of infectious diseases, that is, the properties of a microbial genus or family are described. Each chapter on a given genus or family of bacteria usually includes the following topics (not necessarily in the same order or under the same headings):

1. General characteristics of the members of the genus or family
2. Diseases caused by members of the genus or family
   a. Signs and symptoms of the disease
   b. Pathogenesis and pathology
   c. Virulence factors
3. Laboratory identification procedures
   a. Isolation, cultural, and microscopical characteristics
   b. Biochemical and serological tests
4. Epidemiology
5. Treatment
6. Prevention and control

A generalized discussion of what the reader can expect to find under each heading follows.

GENERAL CHARACTERISTICS

The chapters begin with a generalized description and enumeration of characteristics of the family or genus and the various species within the taxonomic group. Much of the information presented here is in the nature of a preview or summary of what will be spelled out in greater detail in the remainder of the chapter. Included is such information as Gram-

stain reaction, morphology, oxygen requirements, spore formation, encapsulation, cultural characteristics, noteworthy diseases caused by members of the taxonomic group, and properties that are relevant to the group.

DISEASES CAUSED BY MEMBERS OF THE FAMILY OR GENUS OF BACTERIA

Signs and Symptoms

Many of the signs and symptoms (disease manifestations) that are evoked by infectious agents are common to many infectious diseases—signs and symptoms such as headache, malaise, nausea, fever, pain, enlarged lymph nodes, and so on. There may be differences, however, in the combination, degree, sequence, and location of these, depending on the particular infectious agent. There are also diseases in which the signs are very distinctive. This is especially so with the readily observable rashes and lesions of the skin and of the mucosa that occur in some diseases.

The impression sometimes exists that each infectious agent causes only one clinical condition. That is the case with a few microorganisms. Many bacteria, however, are able to cause a number of clinical conditions. The gram-positive cocci are especially noted for the variety of clinical entities in which they may be involved. *Staphylococcus aureus*, for example, in addition to causing infections commonly associated with it, such as boils, bullous impetigo, osteomyelitis, and food poisoning, can give rise to pneumonia, meningitis, cystitis, endocarditis, septicemia, and other disorders. Conversely, bacterial pneumonia may be due to a *Staphylococcus, Streptococcus, Hemophilus, Klebsiella*, or other bacterial agent.

Pathogenesis and Pathology

There is usually an account of how the infectious disease develops that includes the progression of changes in the functioning and architecture of the affected tissues and the microbial factors that are responsible for those changes.

Bacterial Virulence Factors and Other Important Factors

Research has produced voluminous information about the differing toxins, enzymes, antigens, and other products and factors of each bacterial species. Textbooks emphasize the factors that have a role in pathogenesis, identification, epidemiology, and control of a disease.

It is helpful to understand the development and the usage of the terminology that is applied to bacterial factors. Initially a microbial factor often is named according to its activity (for example, fibrinolysin, spreading factor, or hemolysin), or it is named after its discoverer, and these names may remain as the only terms ever used to describe it. Eventually, however, the chemical nature of the factor may be determined. The factor is then given its specific chemical designation. It was eventually learned, for example, that the streptococcal product originally called spreading factor or Duran-Reynals factor is the enzyme hyaluronidase, which dissolves hyaluronic acid, a cementing substance of connective tissue, and which facilitates the dissemination of hyaluronidase-producing bacteria in the tissues. The same substance, in other words, is known under three names: spreading factor, Duran-Reynals factor, and hyaluronidase. This progression in names reflects somewhat the history of the understanding of this factor.

It sometimes happens that one substance produced by a species of bacteria is responsible for several biological activities and the substance remains known under several names. Thus the alpha staphylolysin, the dermonecrotic toxin, and the lethal factor that are produced by *S. aureus* are, insofar as is presently known, all the same substance.

Certain substances are mainly associated with one genus or species of bacteria. Mention oxidase-positive bacteria and the clinical microbiologist thinks first of members of the genus *Neisseria*. Similarly, coagulase is associated with *S. aureus* and lecithinase with *Clostridium perfringens*. These substances often contribute to or are associated with the virulence of the respective bacteria, and they sometimes are useful in the laboratory identification. The association of such substances

with a certain bacterial species does not always mean that some other species do not also produce the same substance.

LABORATORY IDENTIFICATION

A good portion of the time of the clinical laboratory microbiologist is expended in identifying infectious agents in the laboratory.

Overview of the Identification Process

The clinical disease serves as the starting point for the laboratory investigation. The attending physician has a mental list of the microorganisms most likely to cause a given clinical condition. His or her judgments are based on and influenced by such considerations as the patient's age, signs and symptoms, contacts, geographical and dietary history, and predisposing conditions, and what is currently "going around." The culture media to be used, the type of specimen to be obtained, the conditions of incubation, and the tests to be conducted are guided by those clinical judgments.

The first steps in identification usually seek to identify the genus of the isolated bacterium. Next, since most genera contain a number of species with different properties it becomes necessary to identify the species within the genus.

Importance of Identifying Bacterial Strains

It might appear that identifying the microorganism to the species level is all that is ever required. Most species of bacteria, however, through mutation and selection have developed strains that can differ in many respects: virulence, susceptibility to chemotherapeutic agents, ability to metabolize given substrates, and antigenic composition. Often, therefore, one must characterize the microorganism as to its antigenic makeup, susceptibility to chemotherapeutic agents, and other attributes.

Detailed diagnostic information may not always be needed for the clinical management of the individual patient but may be highly important from the public health standpoint. For example, knowing the serogroup of the isolated species often is important for tracing the transmission of the infectious disease agent.

Identification of Nonpathogens

Most of the genera of bacteria studied in medical microbiology contain both pathogenic and nonpathogenic species. Learning the characteristics of the nonpathogens may seem an unproductive pursuit, yet in many instances it is necessary to be able to differentiate the pathogens from the nonpathogens, particularly nonpathogens that constitute the normal microflora. Furthermore, a number of the nonpathogens may opportunistically cause infections in a debilitated individual or under other predisposing conditions.

Laboratory Identification Procedures

Seldom does a single laboratory finding conclusively identify the microorganism. Identification involves a combination of the five general laboratory approaches: microscopical study, cultural examination, biochemical techniques, serological techniques, and animal identification procedures.

As a basic step in laboratory identification a pure culture must almost always be obtained. Pure cultures are obtained by such means as the streak plate, the pour plate, and the use of selective and sometimes differential media.

MICROSCOPICAL CHARACTERISTICS. Gram-staining is routinely performed in the identification of bacteria. It is a process that eliminates those bacteria from the investigation that differ in morphology and staining reaction from the bacterium under scrutiny. Thus if it is determined that the bacterium under study is a gram-negative bacillus, then gram-positive cocci and bacilli and gram-negative cocci are eliminated. Nevertheless there remain hundreds of gram-negative bacilli to consider.

There are situations, however, when the result of the Gram-staining of clinical material, together with a knowledge of the patient's history and symptoms, is enough information to make a presumptive diagnosis as to the causal agent of the disease. The physician with this information may be able to

initiate specific chemotherapy immediately while waiting for definitive laboratory test results. When clinical material is stained, it is important to note the type of host cells on the slide. For example, the presence of large numbers of neutrophils indicates an acute bacterial infection (provided the patient is not receiving immunosuppressive drugs) rather than a viral infection. The following types of clinical material are routinely Gram-stained because the findings may lead to a presumptive diagnosis:

1. Cerebrospinal fluid—for the presence of the causal agent of meningitis.
2. Brain abscess—for infectious bacterial agents, one of which is often an anaerobe belonging to the genus *Bacteroides*.
3. Sputum and transtracheal exudates—for the diagnosis of the causal agent of bacterial pneumonia. The infecting organism can sometimes be seen trapped in pus cells.
4. Urine—for the presence of microorganisms. In properly collected specimens this indicates a urinary tract infection. Catheterized urine is normally sterile.
5. Urethral exudate—from men suspected of having gonorrhea.
6. Other body fluids that normally are sterile—for infectious agents that have a distinctive morphology.

Staining may also be used to identify specific structures on or in the bacterial cell that may be of value in the identification process: capsules, pili, spores, or flagella.

CULTURAL CHARACTERISTICS. The appearance of colonies of bacteria on certain types of media can sometimes be useful in identification. Some bacteria such as *Pseudomonas aeruginosa, S. aureus*, and *Serratia marcescens* produce pigments that are easily visible in broth or semisolid media. Pigmentation is not a confirmatory characteristic of bacteria.

BIOCHEMICAL CHARACTERISTICS. Biochemical and serological tests are the most important tools of the microbiologist in the identification of bacteria. Microbiologists take advantage of the active metabolism of bacteria to identify specific metabolic products. Bacteria exhibit a great diversity in the type of carbon compound they will break down to obtain energy. Many bacterial groups can be differentiated on the basis of their fermentation properties. Fermentation is one of the first bacterial properties tested for, but others are also examined. Some of the biochemical tests employed in the laboratory, including fermentation, are discussed below. It should be noted that 20 or more biochemical tests can now be performed at once using specially designed test kits. They reduce the amount of time for identifying bacteria and also are economical.

1. Fermentation. The fermentative ability of bacteria can be determined by inoculating a suspension of microorganisms into tubes of sterile nutrient broth, each tube containing a different carbohydrate as the carbon source. Some common carbohydrates used are lactose, glucose, maltose, mannitol, and sucrose. If the carbohydrate is used by the bacteria, certain changes in the broth tube can be detected when the medium also contains a pH indicator or an inverted vial. Fermentation of carbohydrates usually results in the production of acid end products. These products reduce the pH of the medium, and a color change can be detected when a pH indicator such as phenol red is present. Phenol red starts to turn yellow at a pH below 6.8. If gas is produced during fermentation, it can be collected in an inverted vial called a *Durham fermentation tube*. Oxidative fermentative media have been developed for the differentiation of microorganisms that use carbohydrates oxidatively rather than by anaerobic fermentation. The agar medium contains peptone, various salts, and bromothymol blue as an acid-base indicator. After sterilization a specific carbohydrate, such as dextrose, is added to two tubes. The test organism is stab-inoculated into the two tubes. One tube is covered with paraffin oil; the second is left open. Acid formation in the open tube indicates oxidative use of the carbohydrate. Acid formation in both tubes indicates fermentation of carbohydrate.

2. Catalase production. The transfer of hydrogen to oxygen in the respiration of some microorganisms results in the production of hydrogen peroxide (H_2O_2). Most aerobic organisms produce an enzyme, catalase, that is capable of oxidizing H_2O_2 to water and oxygen. When colonies on an agar surface are flooded with H_2O_2, bubbles of oxygen will appear if the cells in the colony produce catalase.

3. Hydrogen sulfide production. Some microorganisms are capable of reducing sulfur to hydrogen sulfide (H_2S). One type of medium utilizing high concentrations of the amino acid cysteine and iron salts is inoculated with the test organism. After suitable incubation a black or brown precipitate will appear if the organisms remove sulfur from cysteine and reduce it to H_2S. The precipitate is ferrous sulfide (FeS), formed according to the following reaction:

$$FeSO_4 + H_2S \longrightarrow FeS + H_2SO_4$$

4. Indole production. Breakdown of the amino acid tryptophan by bacteria leads to the accumulation of indole. Indole can be detected in the broth culture by addition of a color reagent called Kovac's reagent. A positive test result is the appearance of a red ring in the broth.

5. Nitrate reduction. The enzyme nitratase can be produced by a wide variety of microorganisms. It is responsible for the catalytic reduction of nitrate (NO_3) to nitrite (NO_2). Nitrite accumulates in the broth culture and can be detected by addition of a special color reagent that reacts with the nitrite to produce a red color. Some bacteria will further reduce NO_2 to nitrogen gas (N_2) or ammonia (NH_3), and if this occurs the nitrite test is apparently negative. The negative tube should be checked for the appearance of unreduced nitrate by the addition of zinc dust besides the nitrite color reagents. The development of a red color confirms the absence of nitrate reduction.

6. Starch hydrolysis. Bacteria cannot assimilate polysaccharides such as starch through the cell wall. Some bacteria, however, are equipped with a special enzyme called *amylase* that is excreted into the surrounding medium when starch is present. This enzyme is capable of hydrolyzing starch into its individual glucose units, which can be assimilated by the bacteria. To test for starch hydrolysis bacteria are first streaked onto a nutrient plate containing starch as the only carbohydrate. Starch hydrolysis is observed by the appearance of a clear zone around bacterial colonies that have been flooded with a solution of iodine. Unhydrolyzed starch is dark blue in the presence of iodine.

7. Gelatin hydrolysis. Gelatin, a protein, can be added to nutrient broth to produce a gel when held at temperatures below 20°C. At temperatures around 25°C gelatin becomes a liquid. Some microorganisms can digest gelatin into its constituent amino acids, thereby reducing the ability of the medium to remain a gel below 25°C. The inoculated tube must therefore be chilled to determine whether gelatin has been hydrolyzed. The enzyme involved in this reaction, *gelatinase*, is excreted into the surrounding medium when gelatin is present.

A considerable number of other biochemical tests are used in the laboratory. Most of them, however, will be discussed only as they apply to specific groups of microorganisms.

SEROLOGICAL CHARACTERISTICS. Bacteria, like many other microorganisms, are antigenic and stimulate the formation of antibodies when injected into test animals. The antigenic potential is due to the chemical nature of surface structures such as cell wall components, capsules, pili, and flagella. By injecting any one of these cellular preparations into a test animal, one can obtain antibodies from the animal's serum. The serum can be used at any time for identification purposes.

When an antigen is tested against its specific antibody, a physical or chemical interaction that is generally observable, such as precipitation or clumping, occurs. No reaction is detectable if there is no specificity between the antigen and the antibody. Antigen-antibody interaction can be used for the purposes of laboratory identification in two ways:

1. Known microorganisms are injected into ani-

mal hosts. Immune sera or antisera specific for each injected microorganism are prepared and refrigerated (they can also be obtained commercially). If at some time an organism is isolated from a patient, the unknown microorganism can be tested against one of the known antisera.

2. Known antigen suspensions of whole cells, pili, flagella, or other structures are prepared in the laboratory. Unknown antisera from infected hosts can be tested against known antigen preparations. A positive reaction with one of the known antigens indicates which microorganisms have infected the host and caused the production of the antibodies. Some of the serological tests used in the clinical laboratory were discussed in the chapters on immunology; others will be discussed in the chapters on specific infectious agents such as the viruses.

ANIMAL IDENTIFICATION. The pathogenicity of an organism can be determined by inoculating it into a susceptible animal species. This is important because many tests do not allow one to differentiate between the pathogenic and nonpathogenic strains of a particular bacterial species. The test animal may die, or various tissues can be examined for signs of disease after a suitable incubation time. This technique has been used to determine the pathogenicity of strains of *Klebsiella pneumoniae* and *Corynebacterium diphtheriae* and others.

EPIDEMIOLOGY

Epidemiologic information, which may include such considerations as age, race, sex, nutritional status, and geographical location, has a bearing on the clinician's level of suspicion as to the causal agent of the patient's disease and indicates the preventive measures that may have to be undertaken for family, contacts, or the public at large. This information also indicates the effectiveness, or the necessity for, immunization and community control measures, and it is a reflection of the disease-causing opportunities and capabilities of a given infectious agent.

TREATMENT

Chemotherapy

Research, experience, and susceptibility testing provide guidelines as to which chemotherapeutic agents are effective against which species and strains of bacteria. It is customary to cite the chemotherapeutic agents of choice and also the alternative agents that may be prescribed if the identified bacterium is resistant to the first-choice chemotherapeutic agent or if the patient has a hypersensitivity or toxic reaction to the first-choice agent.

Passive Immunotherapy

An account of the available passive immunotherapy when appropriate is included. Thus the administration of antiserum (serotherapy) for diseases such as diphtheria or botulism, or the administration of gamma globulin for diseases such as hepatitis A or tetanus, may be advised.

Other Forms of Treatment

Other examples of forms of treatment that may be recommended include surgical intervention, for example, surgical debridement in gas gangrene; enzymatic debridement to speed up removal of pus, fibrin, or edema fluid; and exposure to high concentrations of oxygen (hyperbaric oxygen therapy) for patients who are suffering from certain kinds of anaerobic infections.

PREVENTION AND CONTROL

Active Immunization

Some infectious diseases are completely preventable through active immunization. The type of immunizing material that is prescribed for the disease under discussion is given, that is, whether the immunizing material is a toxoid, an attenuated strain, or a killed suspension. The description includes the immunogenic component of the bacterial cell when it is known (e.g., capsule, cell wall fraction, and so on, that elicits the protective immune response).

Prophylactic Passive Immunotherapy

Prophylactic passive immunotherapy is prescribed for certain diseases, for example, the administration of tetanus antiserum or gamma globulin for someone who has suffered a tetanus-prone injury.

Sterilization and Disinfection Measures

Sometimes it is necessary to point out the sterilization and disinfection measures that are to be employed against the disease agent when these measures differ from those that are routinely used against other bacteria.

STUDY AIDS

REGULARLY CITED BACTERIAL PROPERTIES

The following generalizations make it easier to remember some of the regularly cited properties of bacteria. The description of most bacterial species includes their morphology, grouping and Gram's stain reaction, oxygen requirements, whether they are sporeformers, and whether they are motile.

1. Gram's stain reaction and shape. The Gram's stain reaction and the shape of one genus member apply to all members of the genus. *Hemophilus influenzae* is a gram-negative bacillus; therefore any *Hemophilus* species will also be a gram-negative bacillus. The student is likely to be expected to know the Gram's stain reaction of the major pathogenic bacteria. The sequence in which the bacterial genera are presented in most textbooks is customarily based on their Gram's stain reaction and their shape: gram-positive cocci, gram-negative cocci, gram-positive bacilli, gram-negative bacilli. The mycobacteria and the spirochetes are usually not categorized in those terms. The principal genera of medically important bacteria and their Gram's stain reaction and morphology are as follows:

Gram-positive cocci
- *Staphylococcus*
- *Streptococcus*

Gram-negative cocci
- *Neisseria*

Gram-positive bacilli
- *Corynebacterium*
- *Bacillus*
- *Clostridium*

Gram-negative bacilli
- *Hemophilus*
- *Bordetella*
- *Brucella*
- *Pasteurella*
- *Escherichia*
- *Salmonella*
- *Shigella*
- *Bacteroides*
- *Rickettsia*

2. Endospore formation. The only genera of medical importance in which sporeformers are found are *Clostridium* and *Bacillus*.

3. Oxygen requirements. Most bacteria encountered in an infectious disease are facultatively anaerobic, that is, they are capable of metabolizing under either aerobic or anaerobic conditions. A few genera and species of bacteria are strict or obligate anaerobes. Especially noted for this trait among the bacteria of medical importance are the members of the genera *Clostridium* and *Bacteroides*.

4. Motility. Among the bacteria that are considered in medical microbiology, none of the cocci are motile, all of the spiral-shaped forms are motile, and among the rod-shaped bacteria there is no general rule in respect to motility—some are motile, others are not.

ORGANIZATIONAL AID

As a remembering and organizational aid, the student might well maintain a running table that summarizes the leading information or features of the major bacteria of medical importance. Table 18-1 shows the headings such a table might include (using *Staphylococcus* as an example).

Table 18-1. Format of Suggested Study Guide

Gram's stain, shape	Genus and species	Culture media	Lab tests	Diseases	Other characteristics
+c	*Staphylococcus aureus*	Mannitol salt agar	Coagulase Phage typing	Boils Impetigo Osteomyelitis Food poisoning Scalded skin syndrome	Enterotoxin Hospital-acquired infections Penicillinase Exfoliatin

+c = gram-positive coccus.

TERMINOLOGY

Infectious disease is a pathological condition, and a number of terms and concepts from pathology are used to describe it. An understanding of the following terms will improve the student's comprehension of the discussion of infectious diseases. It is advisable to review the events of inflammation and the cell types that participate in inflammation, antibody formation, cell-mediated immunity, phagocytosis, and allergic reactions that are described in Part IV, Immunology.

ABSCESS a circumscribed accumulation of pus

DIFFERENTIAL BLOOD COUNT an enumeration of white blood cell (WBC) types with the purpose of detecting changes from the normal WBC range (changes in WBC proportions and numbers are brought about by many infectious agents)

EDEMA the accumulation of fluid portions of the blood in tissues; accounts for or contributes to the swelling, blockage, pain, induration, and blanching of the affected site

EMBOLUS a pathological space-occupying aggregate that moves in the vascular system with the constant threat of blocking important vascular channels or spaces (examples: gas bubbles, lipids, pyogenic masses, and, most important, clots [thrombi])

EXUDATE blood components, both fluid and formed (e.g., cells) elements, that accumulate in tissues, spaces, and surfaces (mucosa) as one manifestation of an inflammatory response

ICTERUS jaundice

ISCHEMIA lack of or diminution of blood in a tissue; may be due to blockage, vascular collapse or interruption, pressure, or hemorrhage

LEUKOCYTOSIS temporary increase in white blood cells that may occur in response to injury or disease; may be more specifically termed depending on which WBC type shows an increase: for example, lymphocytosis, an increase in lymphocytes

LEUKOPENIA temporary pathological diminution of white blood cells; may be more specifically termed depending on the cell type involved, for example, lymphopenia, a decrease in lymphocytes

MUCOSA internal epithelial surface lubricated by enzyme-carrying mucus

PARENCHYMA the cells of an organ or tissue that carry out the specific function of that organ or tissue, as opposed to the supporting elements (connective tissue)

PATHOGENESIS the in vivo progression or sequence of events leading to the disease state; the development of the pathological condition

PATHOGNOMONIC (a clinical or laboratory finding that is) so distinctive as to be self-identifying (e.g., the inclusion body elicited in neurons by rabies is so characteristic in appearance that the trained observer knows that this cell was in-

fected by the rabies virus and not some other agent)

PURULENT pus-containing

PYOGENIC pus-inducing, pus-eliciting, pus-generating

PYROGENIC fever-inducing, fever-eliciting, fever-generating

SIGNS disease manifestations that are detectable by an observer; objective indications such as a rash, swelling, and vomiting

SYMPTOMS disease indications felt by the patient; subjective experiences such as headache, malaise

THROMBUS an in vivo blood clot attached in the cardiovascular system (a thrombus or portion thereof may break away to become an embolus [thromboembolus])

ULCER a circumscribed, necrotic, denuded surface area of the skin or mucosa

VESICLE blister; small, circumscribed, elevated area of the skin or mucosa that contains clear fluid

Several combining terms (prefixes and suffixes) are used repeatedly, and an understanding of these helps to interpret the meaning of many words.

PREFIXES

A(N) negative, lack of, deficient, against, reverse of

ADENO pertaining to the glands

ARTHRO pertaining to the joints

ECTO external

ENDO internal

ENTERO pertaining to the intestines

HEMA, HEMATO pertaining to the blood

HYPER above, increased, more than the normal; equivalent to *super*

HYPO below, decreased, less than the normal; equivalent to *sub*

INTER between

INTRA within

MYO pertaining to muscle

OLIGO few

PERI around

POLY many

STOMATO pertaining to the mouth

SUFFIXES

ALGIA pain

DYNIA pain

ECTOMY surgical excision

EMIA a condition of the blood

GENIC causing, eliciting, generating

ITIS inflammation

LYTIC dissolving, causing to flow

OMA tumor

OSTOMY surgical opening

RRHEA flowing, discharge

TROPHIC nourishing

TROPIC turning toward or attaching to

URIA a condition of the urine

SUMMARY

There are a great number and variety of infectious diseases and infectious disease agents. Health care personnel are expected to know the characteristics of the diseases and of the infectious disease agents. Customarily textbook descriptions include the signs and symptoms of the disease, the microbial (virulence) and host factors involved in the development of the disease (pathogenesis), the mode of transmission of the disease, the extent of the population affected in the past (morbidity and mortality statistics), and the laboratory tests and procedures that are used to identify the infecting microorganism. Identification requires knowledge of the cultural conditions and of the components, products, and antigens that are peculiar to a given species. This information is pertinent to deciding the chemotherapy and immunotherapy that is to be applied and to judging the severity of the illness, the prognosis, the expected signs and symptoms in the patient, and the time course of the illness. The availability of vaccines and immunotherapy that

protect individuals and populations are also relevant, as are aseptic and sanitary measures that prevent transmission of the disease agents.

As a mnemonic aid, the chapter includes a condensed version of some of the routinely required basic information about bacteria: Gram's stain reactions, oxygen requirements, spore formation, and motility. Study aids are included: a suggestion of how to organize the information about each species, and definitions of commonly used terms, prefixes, and suffixes that are used in the description of infectious diseases.

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19. THE GRAM-POSITIVE COCCI

OBJECTIVES

To describe the infectious diseases caused by staphylococci

To list the various factors associated with the virulence of *Staphylococcus aureus* and the streptococci

To describe those laboratory tests that differentiate *S. aureus* from *S. epidermidis*

To explain the role of phage typing in the epidemiology of *S. aureus* infections

To briefly describe the techniques for laboratory diagnosis, treatment, and control of staphylococcal and streptococcal infections

To comment on the increasing importance of coagulase-negative staphylococci as causative agents of disease

To describe the laboratory tests used to identify group A streptococci

To list those diseases associated primarily with group B, group C, and group D streptococci

To describe *Streptococcus pneumoniae* in terms of virulence factors, disease associations, and laboratory identification

- The Staphylococci
  - *Staphylococcus aureus*
    - Infections
      - Infections of the Skin and Associated Structures
        - Pyoderma: Abscesses, Furuncles, Sties, Carbuncles, Impetigo
        - Scalded Skin Syndrome.
      - Gastrointestinal Tract Staphylococcal Disease
        - Staphylococcal Enteritis
        - Staphylococcal Food Poisoning
      - Deeper Infections
        - Toxic Shock Syndrome
        - Kawasaki Disease
    - Factors Related to the Pathogenesis of *Staphylococcus aureus* Infections
      - Coagulase
      - Staphylolysins
      - Dermonecrotic Toxin and Lethal Factor
      - Panton-Valentine Leukocidin
      - Staphylococcal Enterotoxins
        - Enterotoxins A, B, C1, C2, D, E
        - Staphylococcal Enterotoxin F
      - Exfoliatin or Exfoliative Toxin
      - Protein A
        - Coagglutination Test
      - Penicillinase
      - Other Substances
    - Epidemiology
      - The Especially Susceptible
      - Phage Typing
      - Antibiograms
    - Laboratory Identification
      - Culture Media
      - Differentiation Between *S. aureus* and *S. epidermidis*
      - Coagulase Test
      - Serological Tests
    - Chemotherapy
      - Drug Resistance
        - To Penicillin and Other Antimicrobials
        - Staphylococcal Tolerance
        - First-Choice and Alternative Drugs
      - Control
        - Interruption of Transmission
        - Attempts to Alter the Carrier State
  - Coagulase-Negative Staphylococci
    - *Staphylococcus epidermidis*
    - *Staphylococcus saprophyticus*
  - The Micrococci
  - The Streptococci
    - General Characteristics
    - Classification
    - Group A Streptococci (*Streptococcus pyogenes*)
      - Streptococcal Antigens
        - Hyaluronic Acid
        - Cell Wall Proteins (M, R, and T)
        - Cell Wall Carbohydrate
        - Cell Wall Peptidoglycan
        - Cytoplasmic Membrane
      - Extracellular Streptococcal Products
        - Erythrogenic Toxin
        - Cardiohepatic Toxin and Nephrotoxin
        - Hemolysins
        - Spreading Factors
      - Pathogenesis
        - Suppurative Diseases
        - Nonsuppurative Diseases
      - Laboratory Diagnosis
      - Treatment and Prevention
    - Group B Streptococci
    - Group C Streptococci
    - Alpha-Hemolytic Streptococci
    - Group D Streptococci
    - Anaerobic Streptococci
    - *Streptococcus pneumoniae (pneumococcus)*
      - General Characteristics
      - Pathogenesis
      - Laboratory Diagnosis
      - Treatment and Prevention

Fig. 19-1. Scanning electron micrograph of microcolonies of *Staphylococcus aureus*. (×14,000.) (Courtesy Z. Yoshii.)

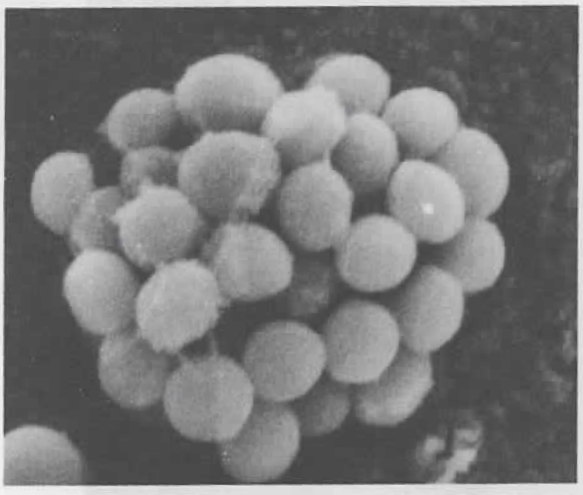
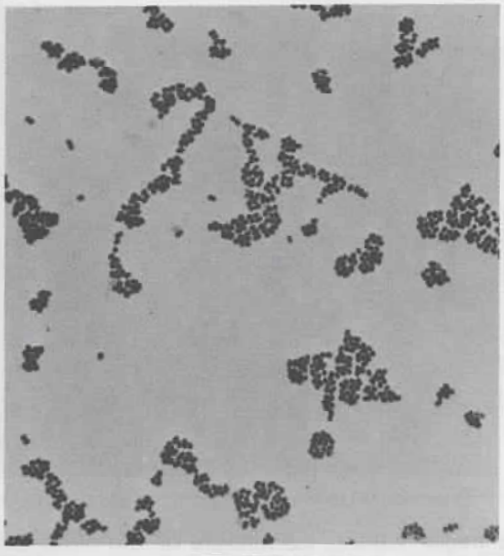


Fig. 19-2. Light micrograph of a smear of *Staphylococcus aureus* taken from a broth culture. (From A. S. Klainer and I. Geis, *Agents of Bacterial Disease*. New York: Harper & Row, 1973.)



Cocci of medical importance are members of the genera *Staphylococcus, Streptococcus*, and *Neisseria*. Staphylococci and streptococci are gram-positive; *Neisseria* are gram-negative. The pathogens can cause infection in virtually any tissue, organ, tract, or system of the body. Since the majority of the infections are acute, the pathogenic cocci are often referred to as the *pyogenic*—pus-eliciting or pus-generating—cocci.

THE STAPHYLOCOCCI

Kloos and Schleifer, using phenotypic character analyses (aerobic acid production from 10 different carbohydrates, coagulase activity, hemolysis, nitrate reduction) and DNA-DNA hybridization studies, classified the members of the genus *Staphylococcus* into at least 16 species. Ten of the species infect humans, but only three are recognized as being clinically important, namely, *S. aureus, S. epidermidis*, and recently *S. saprophyticus*. *S. aureus* is a frank pathogen, the other two being regarded as opportunistically pathogenic. *S. aureus* is the only coagulase-positive (see under Laboratory Identification) human species, so all other human species collectively are referred to as the coagulase-negative staphylococci. *S. hominis, S. haemolyticus*, and *S. simulans* account for a small percentage of infections.

Staphylococci characteristically aggregate in irregular groupings like clusters of grapes (Figs. 19-1 and 19-2). They grow regularly on routinely used laboratory media such as nutrient agar, nutrient yeast agar, soybean-casein digest agar, brain-heart infusion agar, and the broth forms of these agar media. The colonies are usually 2–4 mm in diameter after 24 hours of growth, circular, convex, compact, butyrous (butter-like), and with an entire edge. Staphylococci are aerobic to facultatively anaerobic, nonmotile, and nonsporeforming. They are strongly catalase positive (add hydrogen peroxide to colonial growth and look for the immediate evolution of bubbles of oxygen).

Staphylococci, with *S. epidermidis* and *S. hominis* as the most prevalent and persistent, are among

Fig. 19-3. Carbuncle caused by *Staphylococcus aureus* in a diabetic patient. (From F. H. Top, Sr., and P. F. Wehrle [Eds.], *Communicable and Infectious Diseases* [8th ed.]. St. Louis: Mosby, 1976.)

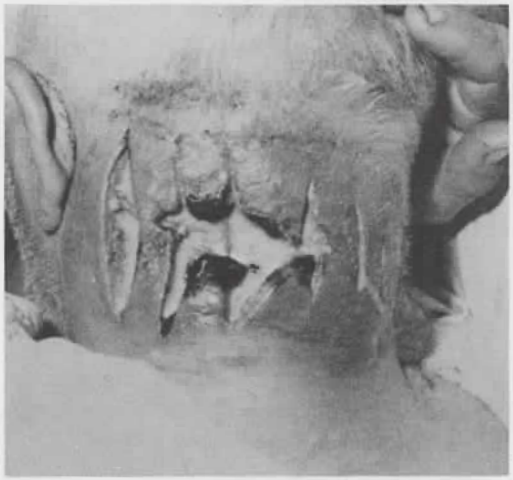


Fig. 19-4. Bullous impetigo caused by *Staphylococcus aureus*. (Courtesy H. Dillon.)

the major natural inhabitants of the skin. Large populations are present in the axillae (armpits) and perineum. *S. haemolyticus, S. warneri*, and *S. capitis* also appear on the skin but usually in small numbers. Staphylococci, especially *S. epidermidis*, are also commonly present in the anterior nares. When a dominant *S. aureus* strain populates the nares, *S. epidermidis* is often greatly reduced. Some staphylococci may also be found in the throat, mouth, vagina, intestinal tract, and mammary glands but in smaller numbers than on skin.

STAPHYLOCOCCUS AUREUS

Infections

The infections and diseases specifically or primarily associated with *S. aureus* are boils, carbuncles, bullous impetigo, scalded skin syndrome, staphylococcal food poisoning, staphylococcal enteritis, staphylococcal osteomyelitis, and toxic shock syndrome.

INFECTIONS OF THE SKIN AND ASSOCIATED STRUCTURES. *Pyoderma: Abscesses, Furuncles, Sties, Carbuncles, Impetigo*. *S. aureus* causes various types of purulent skin infections (pyoderma), the hallmark of which is the abscess. The infection of hair follicles, folliculitis, leads to the formation of furuncles (boils) in the skin or sties in the eyelids. Carbuncles are a deeper, more serious condition that results from tunneling, coalescing abscesses (Fig. 19-3). Either *S. aureus* or *Streptococcus pyogenes* alone or the two agents as coinfectors cause impetigo (Fig. 19-4), a skin infection found primarily in children. Impetigo often begins in the nasal area and spreads from there over the face.

Scalded Skin Syndrome. Scalded skin syndrome (SSS) is an exfoliative dermatitis that encompasses several clinical conditions. It is most often observed in infants and young children. The syndrome usually begins as erythema around the mouth and nose and spreads rapidly to affect the skin of the neck, the trunk, and sometimes the extremities. The epidermal necrolysis, mediated by the toxin exfoliatin, results in extensive areas of denuded skin as sheets of the overlying epidermis loosen. Transudates and exudates are not copious

and fatalities are infrequent since the condition is largely limited to the superficial layer of the skin.

GASTROINTESTINAL TRACT STAPHYLOCOCCAL DISEASE. *Staphylococcal Enteritis*. Patients who have received chemotherapeutic agents, especially via the oral route, may develop staphylococcal enteritis. In staphylococcal enteritis the chemotherapeutic agents typically reduce the normal intestinal microflora, thereby giving an endogenous *Staphylococcus* the opportunity to multiply virtually unrestrictedly in what is termed a superinfection.

Staphylococcal Food Poisoning. Staphylococcal food poisoning is one of the most prevalent types of food poisoning. In the strict sense, this is not an infection. Certain strains of staphylococci excrete into foods the heat-stable exotoxin called *enterotoxin*. When ingested with the food, the enterotoxin causes a type of food poisoning that is accompanied by vehement vomiting (projectile vomiting), cramps, diarrhea, and prostration 2–8 hours after ingestion. Exterotoxin food poisoning is rarely fatal. The patient is fully recovered in 24–48 hours. Many foods support the growth of staphylococci, particularly those that contain a large amount of protein: custard, deviled eggs, cream, salad dressings, and ham. After the bacteria are introduced into the food, there must be adequate conditions for incubation so they can multiply and shed enterotoxin into the food. Food preparers and handlers with staphylococcal skin lesions, especially on the hands, and nasopharyngeal carriers are the most likely to contaminate the food. Unrefrigerated or inadequately refrigerated foods allow for the necessary incubation conditions. (See boxed essay, p. 449.)

DEEPER INFECTIONS. Extensive and deeper infections may be primary infections or may stem from an infection that has metastasized from a cutaneous infection or from a carrier site. Serious staphylococcal infections are not likely to occur in healthy individuals but rather in those who have a precondition such as extensive surgery, burns, diabetes, cystic fibrosis, lower respiratory tract viral infection, or decubitus ulcers, and in those who are immunosuppressed or immunodefective, especially in some aspect of their phagocytic system (see Immunodeficiencies in Phagocytes, Chapter 17). *S. aureus* can cause infections in many tissues and organs: endocarditis, meningitis, pneumonia, cystitis, septicemia, infection of postoperative wounds, and others.

Toxic Shock Syndrome. Toxic shock syndrome (TSS) is a multisystem, febrile illness with abrupt onset and with hypotension (shock). Vomiting, diarrhea, a diffuse macular erythematous rash with subsequent palmar and plantar desquamation (peeling of the skin on the palms of the hands and soles of the feet), and hyperemia of mucous membranes regularly occur. Disseminated intravascular clotting is an occasional complication. Death may be caused by profound shock, respiratory failure, and disseminated intravascular coagulopathy. The abrupt onset of TSS in the absence of a clinical prodrome suggests that a toxin is involved. It is believed that the toxin responsible for the illness is an enterotoxin-like protein called *staphylococcal enterotoxin F*.

TSS was first described as a syndrome in 1978, but it is likely that cases were seen before then. TSS primarily is reported in young white women who are menstruating at the time of onset and who use tampons. As of June 15, 1983, there had been 2204 cases of TSS reported, 96 percent of which were in women. Ninety percent of the cases in women were associated with menstruation and 99 percent of the menstruation-associated cases occurred in tampon users. TSS not associated with menstruation has been reported after childbirth and therapeutic abortions and from infected abrasions, abscesses, burns, insect bites, surgical wounds, sweat glands, and lymph nodes. Since 1980 there has been a decrease both in the number of menstruation-associated cases and in the total number of TSS cases per year. The case fatality rates were 10 percent in the pre-1980 period and have been around 3 percent since then.

Kawasaki Disease. Kawasaki disease, the cause of which is unknown, is described as a mucocutaneous lymph node syndrome that occurs mainly in children below the age of 5. It appears to be related to TSS because of many similarities: fever that is

unresponsive to chemotherapy, rash and subsequent desquamation, enlarged cervical lymph nodes, mucosal changes, and other clinical signs similar to those seen in TSS.

Factors Related to the Pathogenesis of Staphylococcus aureus *Infections*

S. aureus strains may produce as many as 25 to 30 proteins, some of which may be associated with pathogenesis. The factors that are either specifically or primarily related to *S. aureus* are coagulase, staphylolysins, dermonecrotic toxin, lethal factor, Panton-Valentine leukocidin, staphylococcal enterotoxin, staphylococcal enterotoxin F, protein A, and penicillinase.

COAGULASE. Coagulase, a plasma-clotting protein, although produced by some other bacteria, is almost exclusively associated with *S. aureus*. Staphylococcal coagulase occurs in two forms, bound and free (or extracellular). Bound coagulase, also called *clumping factor*, is found on the surface of staphylococci. Staphylococci with bound coagulase on their surface clump together in plasma.

Free coagulase clots plasma. This phenomenon is the basis for the laboratory-conducted coagulase test (see under Coagulase Test). The production of free coagulase correlates well with the virulence of *S. aureus* (so does production of staphylolysins, leukocidins, nuclease, and other factors). This is not to say that coagulase is the sole or necessarily the major virulence factor of *S. aureus*, as is quite commonly believed. How coagulase serves as a virulence factor can only be surmised. Probably the staphylococci are protected from the natural immune mechanisms of the host because of the organism's ability to induce the host to surround the bacterial cells (or the tissues where they are located) with deposits of fibrin.

STAPHYLOLYSINS. Staphylolysins are hemolysins that are categorized among the staphylococcal exotoxins. They also are cytotoxic for cells other than red blood cells. They are of several types: alpha, beta, gamma, and delta. There are antigenic differences among them, differences in the species of red cells that they are able to lyse, differences in the cations that are required for their activation, differences in the cell membrane substrates on which they act, and differences in their toxic activities. The alpha lysin lyses rabbit red blood cells; the delta lysin lyses human, sheep, rabbit, horse, mouse, rat, and guinea pig red blood cells. More than 90 percent of the staphylococcal strains that are pathogenic for humans produce a combination of the alpha and delta lysins. Alpha and delta staphylolysins also serve as the dermonecrotic toxin and the lethal factor of *S. aureus*. They are, moreover, leukotoxic.

DERMONECROTIC TOXIN AND LETHAL FACTOR. Staphylolysin-containing filtrates from cultured staphylococci bring about necrosis when injected into the skin, and death when injected intravenously into laboratory animals.

PANTON-VALENTINE LEUKOCIDIN. Many *S. aureus* strains produce a leukocidin different in action and composition from the leukotoxic staphylolysins. Leukocidin increases the permeability of leukocytes to cations and so leads to the swelling and rounding up of the cells. The membranes of cytoplasmic granules fuse with the cytoplasmic membrane of the cell, causing release of the cytoplasmic granules and cell disruption.

STAPHYLOCOCCAL ENTEROTOXINS. *Enterotoxins A, B, C1, C2, D, E.* There are six (A, B, C1, C2, D, E) chemically and immunologically related staphylococcal enterotoxins. Approximately one-third of *S. aureus* strains, under suitable conditions of incubation, shed an exotoxin, termed *staphylococcal enterotoxin*, into food. When ingested with the food, the toxin causes the most prevalent type of food poisoning. The toxin can withstand 30 minutes of boiling (is thermostabile) and is not inactivated by the digestive enzymes.

Staphylococcal Enterotoxin F. Staphylococcal enterotoxin F (SEF) is an enterotoxin-like protein that is found in almost all *S. aureus* strains isolated from individuals suffering from toxic shock syn-

INTERSTATE COMMON-SOURCE OUTBREAK OF STAPHYLOCOCCAL FOOD POISONING

On August 4, 1982, at least 51 cases of acute gastrointestinal illness occurred among 248 passengers on an Ohio River boat trip from Pittsburgh, Pennsylvania, to Waterford Park, West Virginia. Food had been provided by an Allegheny county caterer. Symptoms included diarrhea (86 percent), nausea (81 percent), vomiting (77 percent), and abdominal cramps (61 percent). The incubation period was 1½–7½ hours. Twenty-six of those affected sought medical aid at an Ohio hospital; three were hospitalized. Food histories of 199 persons interviewed implicated sliced ham as the vehicle of transmission. The attack rate was 30 percent (51/171) for those who ate ham and 0 percent for those who did not eat ham. *S. aureus* phage type 53 was isolated from stool specimens from eight patients treated at the hospital. Allegheny County Health Department tests on specimens from a previously unopened cryovac-packaged ham obtained August 5 showed *S. aureus* phage type 53.

After other outbreaks, the U.S. Department of Agriculture tested previously unopened cryovac-packaged hams and found 12 of 21 hams culture positive for *S. aureus*. The isolates from nine of these hams were phage type 53; none had preformed enterotoxin, but the isolates were enterotoxin type A–producing. On August 17, the USDA formally recalled 36,000 pounds of ham distributed by the Brooklyn, New York, processor to five states (Connecticut, Maryland, New York, Pennsylvania, and Virginia). USDA inspection implicated a "cold smoke" method of processing the specialty ham, allowing exposure to bacterial growth temperatures for over 6 hours. The processor has now discontinued this method.

from *MMWR* 32:183, 1983.

drome. The TSS strains predominantly belong to phage group I, types 29 and 52 (see Phage Typing later in this chapter). About 90 percent of healthy individuals have antibodies against SEF, whereas TSS patients either do not have antibodies or have them at very low levels.

EXFOLIATIN OR EXFOLIATIVE TOXIN. Phage group II strains of *S. aureus* produce a toxin, which is controlled by a plasmid or a chromosome or both, that is involved in scalded skin syndrome.

PROTEIN A. Protein A is a surface component of most *S. aureus* strains. It is linked to the peptidoglycan layer of the cell wall, but some of it is released extracellularly. Protein A has the unusual property of binding nonspecifically to the Fc portion of IgG (except to subclass IgG3) in normal serum. Note that we usually find a protein such as protein A serving as an antigen, and if so it is the other end of the Ig molecule, the Fab portion, that we normally expect to bind to the protein, not the Fc portion as here. (Protein A can serve as an anti-

gen and as such does interact with the Fab portion of specific antibody.) Protein A, because of this property of binding to Fc, is believed to be antiphagocytic by competing with neutrophils for the Fc portion of specific opsonins. Protein A has other biologic effects: It elicits hypersensitivity and inflammation, it injures platelets, and it prevents the absorption of bacterial viruses (bacteriophages) that are specific for *S. aureus* (these viruses are called *staphylophages*).

Coagglutination Test. A cleverly designed serological test, the coagglutination test is based on the unusual property of protein A to bind nonspecifically to the Fc portion of IgG. Antibodies that are specific for other bacteria such as streptococci, salmonellae, neisseriae, and *Hemophilus* are allowed to couple via the Fc portion of the antibody molecule to protein A that is still bound to killed staphylococci. This leaves the antigen-specific end of the molecule, the Fab end, free to attach to the bacteria for which the Fab is specific. When bacteria for which the Fab is specific are added, a lattice is formed that is visible as a coagglutinate (clumps of the staphylococci and the test bacteria).

PENICILLINASE. *S. aureus* is noted for its resistance to certain of the penicillins. Many strains produce the enzyme penicillinase, which affects the beta-lactam portion of the penicillin molecule (see Fig. 11-2). The enzyme is therefore termed a *beta-lactamase*. Plasmids, which can be transferred via bacteriophages, or penicillin determinants on the chromosome code for the production of penicillinase.

OTHER SUBSTANCES. Staphylococci produce other enzymes and other substances that contribute to their virulence, metabolic activities, and antigen composition: hyaluronidase, staphylokinase, lipase, proteinase, thermostable nucleases (DNase and RNase), and a capsule in some strains.

Epidemiology

Staphylococci are among the hardiest of the vegetative bacteria. They are found on the skin and mucous membranes of humans and so are readily deposited on items that can serve as fomites (towels, sinks, drawer pulls). There is a comparatively high carrier rate—about 30 percent in the general population, and considerably higher in certain segments of the population, notably among hospital personnel. The principal carriage site from which staphylococci are disseminated is the anterior nares. The perineum is another common carriage site. With so high a carrier rate, the source of infection may well be an individual's own microflora. (See boxed essay, p. 451.)

THE ESPECIALLY SUSCEPTIBLE. Particularly susceptible to serious staphylococcal infections are the newborn and those in a debilitated condition: the aged, the chronically ill, persons with metabolic disorders (classically diabetics), and persons with traumatic or surgical wounds. The newborn and the debilitated—the susceptible—are those most likely to be exposed to the group with the high carrier rate, namely, nurses, surgeons, and other hospital personnel. The obligation of hospital personnel to carry out scrupulous medical asepsis is obvious. Patients entering the hospital for the treatment of other infections may have their drug-susceptible staphylococci suppressed by chemotherapeutic agents and replaced by drug-resistant staphylococci.

PHAGE TYPING. At one time there was an alarmingly high incidence of nosocomial (hospital-associated) staphylococcal infections, and the chain of transmission was not readily evident. Hospital-associated staph infections still occur, but now the sources of the microorganism frequently can be traced by a laboratory procedure known as *phage typing*. Once the source of the infectious agent is determined, measures can be taken to break the chain of transmission. Phage typing makes it possible to identify various *Staphylococcus* strains. The procedure is applicable to a number of other bacterial species and is a useful epidemiological tool. Phage typing for epidemiological purposes is best known in relation to "hospital staph" and will therefore be explained here in some detail. *Phage*

A STUDY OF *STAPHYLOCOCCUS AUREUS* COLONIZATION IN CHRONIC CARE PATIENTS

S. aureus upper respiratory tract colonization was studied in 326 chronic care patients over a period of 24 years. The number of *S. aureus* strains per patient ranged from one to eight, with an average of 2.8. Each patient had a predominant strain that tended to be permanent, with 87 percent persistence over the years that a given patient was studied (average number of years each patient was studied was 4.6). The average number of strains did not increase but remained at a level of two to four strains per patient. This suggests that the acquisition of additional strains was blocked by resident strains, an example of interference. Antibiotic therapy usually made no permanent changes in the carrier state of long-term patients or in the specific strains colonizing these patients.

From C. H. Zierdt, *J. Clin. Microbiol.* 16:517, 1982.

is an abbreviation for *bacteriophage*, which literally translates to "bacteria eater." Bacteriophages are viruses that infect and in many instances annihilate by lysis (virulent phage) the bacteria they infect. Bacteriophages can be named more specifically after the bacteria they infect. Thus the bacterial viruses that infect *Escherichia coli* are termed *coliphages*, those that infect staphylococci are termed *staphylophages*, and so forth.

To understand phage typing it is necessary to know that strains of bacterial viruses are specific for strains of bacteria within a bacterial species. Not all staphylophages can lyse every strain of *S. aureus*. Certain strains of phage will cause lysis of only certain strains of *S. aureus*. The staphylophages have been assigned numbers, and what are termed the *basic typing phages* have been organized into lytic groups in an internationally agreed-on set of basic typing phages (Table 19-1).

The laboratory procedure in phage typing involves applications of the various staphylophage types to staphylococci growing on an agar medium surface. The staphylococci to be typed are spread on an agar medium surface so as to give rise to confluent growth (a "lawn" of growth). The plate is marked off in small squares—a grid. A drop of each phage type is then placed on the inoculated agar surface. The drops are spotted on appropriately identified squares. Plaques (holes in the bacterial lawn) appear in the squares where the applied phage is lytic for the test staphylococci (Fig. 19-5). A phage typing investigation of this sort might reveal that staphylococci isolated from a surgical wound have the same phage pattern as staphylococcal isolates from the attending surgeon, from surgical technicians, or from attending nurses.

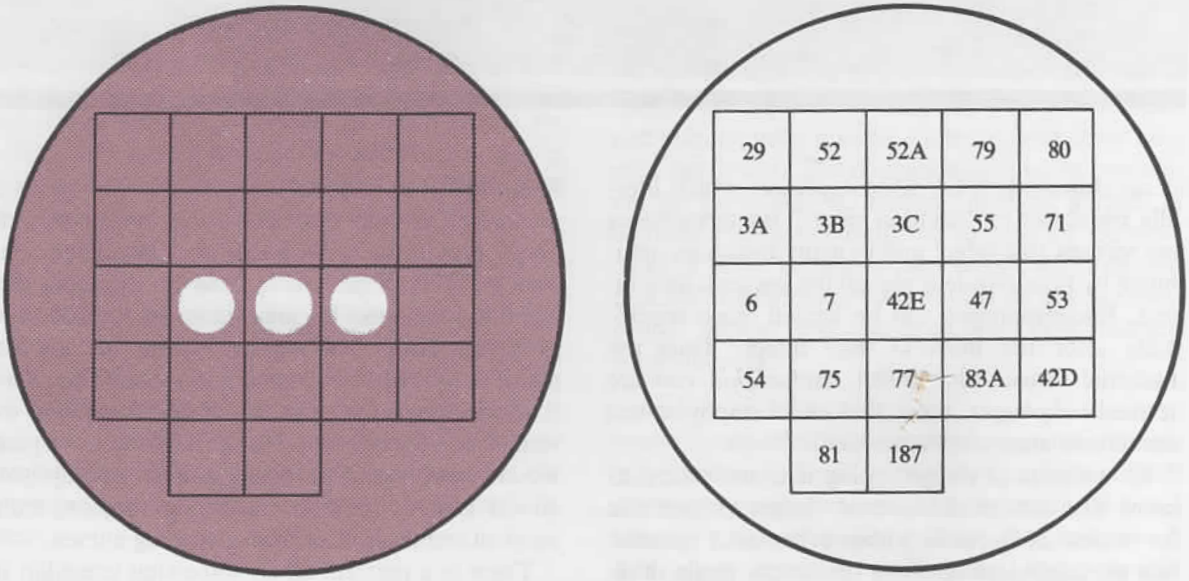
There is a pattern, albeit somewhat irregular, in which certain staphylococcal diseases are found to be related to certain phage types of a lytic group. Thus phages in lytic group I are most often involved in hospital-associated infections, and enterotoxin-producing strains of staphylococci are usually members of lytic groups III and IV.

ANTIBIOGRAMS. Similar or identical patterns of resistance to antibiotics (antibiograms) can also be used as presumptive evidence of the relatedness of strains in epidemiological tracing.

Table 19-1. Lytic Groups of *Staphylococcus* Typing Phages

Lytic group	Staphylophages within group								
I	29	52	52A	79	80				
II	3A	3B	3C	55	71				
III	6	7	42E	47	53	54	75	77	83A
IV	42D								
Not allotted			81	187					

Fig. 19-5. Phage typing of staphylococci. An agar plate is marked off in squares. The plate is then streaked with *S. aureus* and allowed to dry. Specific phage suspensions, corresponding to the strains indicated on the right, are placed in the appropriate squares. After being allowed to dry again, the plate is incubated for 12–18 hours. The plate on the left shows confluent growth except where plaques appear in the squares corresponding to phage preparations 7, 42E, and 47.



Laboratory Identification

The routine clinical laboratory identification of staphylococci poses one of the less difficult bacterial identification problems. It may be necessary first to differentiate among the gram-positive cocci, namely staphylococci, streptococci, and micrococci. Differentiation from the streptococci is done by means of the catalase test, colony characteristics, type of blood agar plate hemolysis, and microscopical characteristics. Differentiation from micrococci is described under The Micrococci.

Once it is established that the isolate is a *Staphylococcus*, most clinical laboratories go no further than to differentiate *S. aureus* and *S. epidermidis*, although there are more and more instances when laboratories seek to discern if the isolate is a coagulase-negative *Staphylococcus*, especially the species *S. saprophyticus*. The other important clin-

ical laboratory procedure is the testing of the isolate for antimicrobial susceptibility.

CULTURE MEDIA. When contamination by other organisms is likely to be minimal (for example, in a specimen from a boil), blood agar containing sheep red blood cells is the recommended medium. A selective medium is employed when contamination by other organisms is expected to be heavy, as in a fecal specimen from a patient with a suspected staphylococcal enteritis. *S. aureus* and *S. epidermidis* tolerate high concentrations of salt (7.5 to 10%) and they are relatively resistant to polymyxin, phenylethyl alcohol, and tellurite, and so selective media that contain these substances are employed in the isolation of staphylococci.

One of the better-known selective media used for staphylococcal isolation and identification is mannitol salt agar (MSA). It contains a high concentration of NaCl, 7.5% (about 0.5% is the usual concentration in culture media), which is extremely inhibitory to most other bacteria. In addition to being a selective medium, MSA is a differential medium. The carbohydrate mannitol and a pH indicator, phenol red, serve as its differential components. The medium surrounding a mannitol-positive colony turns from the typical red color of the indicator to a yellow color when acid is produced from the fermentation of mannitol. Mannitol fermentation under anaerobic conditions differentiates *S. aureus* from *S. epidermidis*.

Other media that are used in conjunction with the staphylococci, but not ordinarily in routine identification, include egg yolk agar for the detection of lipase, and DNA-containing medium for the detection of DNase activity.

DIFFERENTIATION BETWEEN *S. AUREUS* AND *S. EPIDERMIDIS*. Pigment production, though indicatory, is not a reliable criterion for distinguishing between *S. aureus* and *S. epidermidis* because pigment production is not a totally stable characteristic. Coagulase-positive human strains are regarded as *S. aureus* (Table 19-2). Coagulase positiveness is considered by most laboratories as the definitive identifying characteristic of *S. aureus*. Mannitol fermentation and blood agar plate hemolysis contribute to distinguishing between the two species.

COAGULASE TEST. There are several laboratory procedures for determining coagulase production: the tube test, the slide test, and a test performed with plasma incorporated into an agar medium. Plasma is used in all of the tests. Plasma is the fluid portion of the blood that contains the elements required for clotting (serum is the fluid portion minus the clotting factors).

The conventional coagulase test is the tube test. The test bacteria are suspended in citrated or oxalated plasma (rabbit plasma is the preferred animal plasma). The inoculated plasma is incubated and examined periodically for clot formation. Clotting usually occurs within a range of a few minutes to several hours, but the test is observed for 24 hours.

SEROLOGICAL TESTS. Serological tests are of limited usefulness and are not part of routine laboratory identification of *S. aureus*. The detection of antibodies to cell wall teichoic acids of *S. aureus* correlates with the presence of staphylococcal infective endocarditis or deep tissue infection.

Chemotherapy

Superficial infections in an otherwise healthy person require no chemotherapy; treatment consists of drainage, application of moist heat, and immobilization. Deep-seated infections require intensive, prolonged chemotherapy. One of the most difficult of the deep-seated infections to eradicate is infective carditis. A multidrug regimen is recommended.

DRUG RESISTANCE. *S. aureus* is very adaptable in its ability consistently to develop resistance to many antimicrobials. The corollary to that is that any isolated *S. aureus* (and for that matter *S. epidermidis* also) should be promptly assayed for susceptibility or resistance to antimicrobials.

To Penicillin and Other Antimicrobials. The majority of *S. aureus* strains were sensitive to penicillin when penicillin was first introduced in 1940. Now, however, many of the strains, through the

Table 19-2. Properties That Differentiate *S. aureus* from *S. epidermidis**

Property	*S. aureus*	*S. epidermidis*	Comments
Colony pigmentation	Gold	White	A preliminary presumptive property in laboratory differentiation
Coagulase production	Positive	Negative	The principal criterion for laboratory differentiation
Anaerobic fermentation of mannitol	Acid produced	Negative	The second most important criterion for routine laboratory differentiation
Production of staphylolysins	Positive	Negative	Staphylolysins produce the beta type of blood agar plate (BAP) hemolysis; BAP hemolysis is a helpful but secondary criterion for routine laboratory differentiation
Type of cell wall teichoic acid	Ribitol (polysaccharide A)	Glycerol (polysaccharide B)	A major antigenic component of the cell wall; receptor site of phage
Protein A in cell wall	Present	Absent	Protein A is antiphagocytic, has anticomplement activity, causes injury to platelets; group antigen of most *S. aureus* strains
Toxins specifically associated with staphylococci	Panton-Valentine leukocidin; staphylococcal enterotoxin; exfoliatin (epidermolytic toxin)	Absent	—
Lipase	Positive	Negative	Lipases act on lipids of the skin and explain invasion of the sebaceous glands
Heat-resistant endonuclease	Positive	Negative	

*The first four properties are used in routine laboratory differentiation.

plasmid-mediated production of penicillinase (beta-lactamase), have become resistant to penicillin. Many of the penicillin G–resistant class of isolates furthermore exhibit multiple drug resistance to such drugs as cephalosporins, aminoglycosides, macrolides (erythromycin, oleandomycin), and lincosamides (lincomycin, clindamycin). There is an increasing nosocomial prevalence of resistance to methicillin, a penicillinase-resistant penicillin.

Staphylococcal Tolerance. There is another form of staphylococcal antimicrobial resistance reported in recent years that is termed *tolerance*. Low concentrations of antimicrobials prevent the multiplication of tolerant strains, that is, the minimal in-

hibitory concentration is low, but the minimal bactericidal concentration is unusually high. Strains tolerant to the bactericidal effect of one drug, for example, of nafcillin (or methicillin), furthermore often exhibit cross tolerance to other antimicrobials.

FIRST-CHOICE AND ALTERNATIVE DRUGS. The drug of first choice for non-penicillinase-producing *S. aureus* is penicillin G or V; for penicillinase-producing strains a penicillinase-resistant penicillin (for oral administration cloxacillin or dicloxacillin; for parenteral administration methicillin, nafcillin, or oxacillin). Alternative drugs, especially for those with a penicillin allergy, include cephalosporin, clindamycin, and vancomycin.

Control

The control of staphylococcal infections includes measures to interrupt transmission and attempts to suppress the carrier-state population of staphylococci by the administration of topical antibiotics. The antigens responsible for inducing immunity to *S. aureus* have not been identified, so routine immunization is not available.

INTERRUPTION OF TRANSMISSION. Interruption of transmission in a family in which members experience repeated staphylococcal infections begins with scrupulous hygienic practices. In hospitals persons with lesions should not be allowed to come in contact with susceptible patients. Carriers should be traced by such means as phage typing when hospital outbreaks occur. Since nursery and surgery patients are especially vulnerable to staph infections, hospital personnel in these sections must be particularly diligent about aseptic practices.

ATTEMPTS TO ALTER THE CARRIER STATE. It has not been possible to eliminate permanently the principal reservoir of staphylococci, the nasal carrier. Topical applications of neomycin, bacitracin, and gentamicin ointments for protracted periods of time, especially for those who repeatedly experience staphylococcal infections, reduce the numbers of cultivable organisms. Some type of rotation in the use of antibiotics is suggested so that the selection pressure for resistant strains is periodically changed.

COAGULASE-NEGATIVE STAPHYLOCOCCI

Coagulase-negative staphylococci (CONS) are gram-positive cocci appearing in clusters that are catalase positive, nonhemolytic on 5% sheep blood agar, and coagulase negative. They are rather ubiquitous, and so usually they are adjudged to be contaminants when they appear on culture media. There is an increasing interest in CONS, however, because of the number of reports that document them as causal agents in urinary tract infections, bacteremia, and infections of prosthetic devices, indwelling catheters, vascular grafts, and wounds. The two best known species are *S. epidermidis* and *S. saprophyticus*.

Staphylococcus epidermidis

S. epidermidis is a major skin inhabitant. It is the organism most frequently associated with CONS endocarditis, colonization of prostheses, bacteremia, wound infections, and urinary tract infections in elderly hospitalized men.

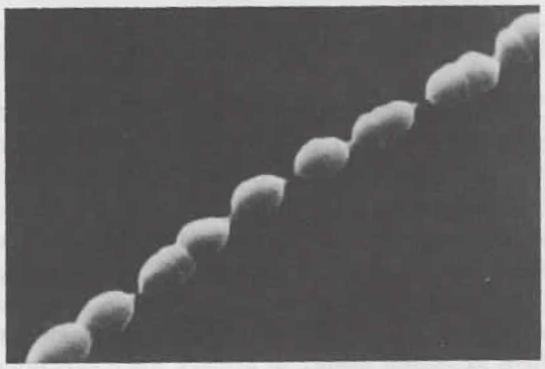
Staphylococcus saprophyticus

S. saprophyticus has a predilection for the urinary tract, especially in young women, which suggests that hormonal factors may be involved. *S. saprophyticus* is novobiocin susceptible, and this is a major and reliable characteristic in differentiating *S. saprophyticus* from *S. epidermidis*.

THE MICROCOCCI

Members of the genus *Micrococcus* bear certain resemblances to staphylococci: micrococci are gram-positive cocci, the colonies are similar to staphylococcal colonies, and micrococci may be

Fig. 19-6. Scanning electron micrograph (×10,000 before 55% reduction) of *Streptococcus pneumoniae*. (From S. A. Klainer and I. Geis, *Agents of Bacterial Disease*. New York: Harper & Row, 1973.)



transients on the skin and mucous membranes. Micrococci are widely distributed in soil and fresh water and are commonly recovered from environmental samples. Species include *M. luteus*, *M. roseus*, and *M. varians*. In rare instances they cause endocarditis. They may appear in specimens as contaminants and must therefore be differentiated from other gram-positive cocci. Micrococci are distinguished from staphylococci by the former's lack of susceptibility to lysis by lysostaphin, by their failure to ferment glucose anaerobically, and by their failure to produce acid from glycerol when grown in the presence of erythromycin.

THE STREPTOCOCCI

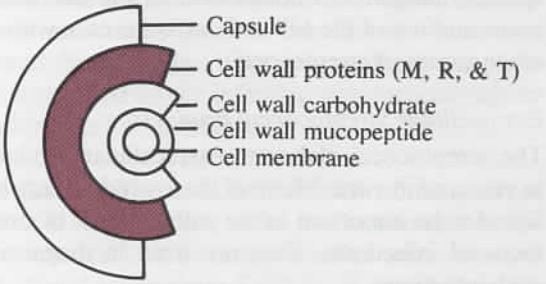
GENERAL CHARACTERISTICS

The streptococci are gram-positive cocci that appear in chains or clusters (Fig. 19-6). In the laboratory streptococci grown on enriched media may produce much longer chains than those seen in clinical specimens. Most species are facultative anaerobes, although one group that inhabits the intestinal tract and female genital tract is made up of strict anaerobes. The virulent species may produce a hyaluronic acid capsule as well as the surface antigen called *M protein*. The streptococci are fastidious in their nutritional demands, often requiring many amino acids and vitamins as well as purine and pyrimidine bases. The primary habitat of the streptococci is the upper respiratory tract of humans. Streptococcal disease in humans includes pharyngitis, scarlet fever, impetigo, rheumatic fever, pneumonia, meningitis, and other pathological conditions. Streptococci also cause mastitis in cows and can therefore be found in milk used for human consumption, again indicating the importance of pasteurization to prevent human disease.

CLASSIFICATION

At one time the streptococci were classified according to the diseases they produced. This system was found to be totally unreliable, and differentiation was later based on the hemolytic behavior of streptococcal strains on blood agar. According to this classification the streptococci are separated into three groups: alpha, beta, and gamma. Most of the alpha-hemolytic species are members of a group referred to as the *viridans* group (*viridans* specifically refers to alpha-hemolytic streptococci without a defined, specific capsular polysaccharide) and are found primarily in the upper respiratory tract. The beta-hemolytic streptococci are the most important group because they contain the major human pathogens. Some beta-hemolytic streptococci are, however, not pathogenic. The gamma-hemolytic or nonhemolytic streptococci are a heterogeneous group and are not considered primary pathogens. Many are found as commensals in humans and animals. Although hemolytic activity is a good presumptive criterion for differentiation, it is not suitable for determining pathogenicity, and immunological differences must be used. Rebecca Lancefield and her co-workers developed a technique for differentiation of the streptococci. They placed them into serological groups A through O based on the antigenic characteristics of a cell wall carbohydrate called the *C substance*. The main pathogenic groups are A, B, C, D, and G, and each group is given a species name. Some

Fig. 19-7. The antigenic components of the streptococcal cell envelope. Rings portray relative position but not size of components. The capsule is not immunogenic.



groups have but a single species. More than 90 percent of streptococcal disease in humans is caused by group A beta-hemolytic streptococci. This group has been given the species name *Streptococcus pyogenes*. Group B streptococci are named *S. agalactiae*; group C, *S. zooepidemicus*; Group D, *S. faecalis*. Each group can be further separated into serological types according to the antigenic characteristic of certain cell wall proteins: the M, R, and T proteins.

GROUP A STREPTOCOCCI (*STREPTOCOCCUS PYOGENES*)

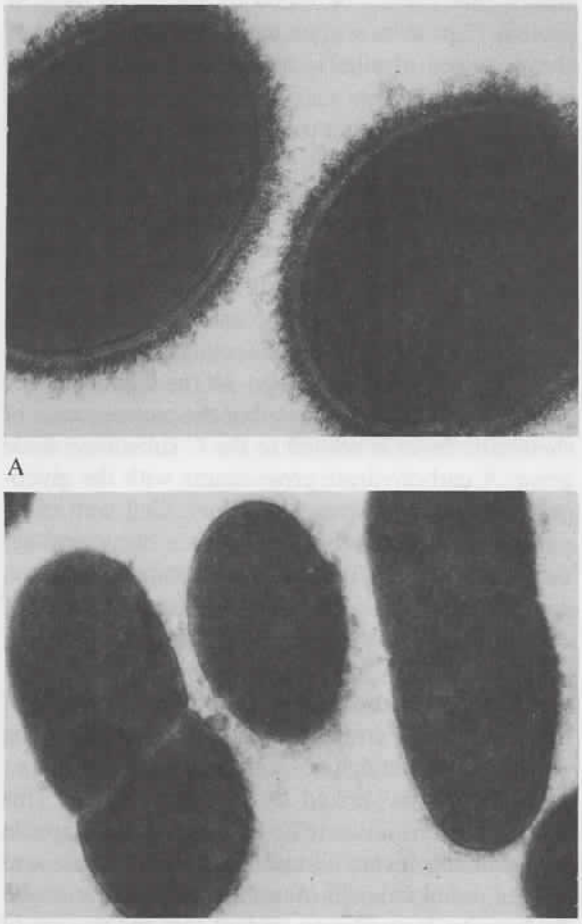
Streptococcal Antigens

The antigenic components of the streptococcal cell wall are shown in Figure 19-7. Their importance in pathogenesis and classification will now be described.

HYALURONIC ACID CAPSULE. Hyaluronic acid is a capsular material produced primarily by streptococcal species in groups A and C. Hyaluronic acid is not immunogenic, which appears to be related to the fact that it is also a prominent component of mammalian connective tissue. The hyaluronic acid capsule is an antiphagocytic substance that disappears during the later stages of the streptococcal growth cycle.

CELL WALL PROTEINS (M, R, AND T). The M protein of the streptococcal cell wall is the major virulence factor of group A beta-hemolytic streptococci (Fig. 19-8). It enables the bacterial cell to inhibit phagocytosis. Cells from which the M protein has been removed lose their antiphagocytic ability. Antibodies produced in a patient to a type-specific M protein have been shown to confer long-lasting

Fig. 19-8. Electron micrograph (×84,000 before 33% reduction) of cross sections of *Streptococcus pyogenes* with M protein (A) and without M protein (B). M protein appears as pilus-like appendages on the bacterial surface. (From E. H. Beachey and G. H. Stollerman. In D. C. Dumonde [Ed.], *Infection and Immunology in the Rheumatic Diseases*. Oxford, England: Blackwell, 1976.)



A

B

immunity against infection from that streptococcal strain. On the basis of differences in their M protein, the members of group A streptococci have been separated into more than 60 different types. Theoretically you could expect 60 different *S. pyogenes* infections in your lifetime before you were completely immune to *S. pyogenes*.

The T protein provides a system of serotyping strains that cannot be typed by means of their M protein. T protein is often used for identification of streptococcal strains isolated from patients with impetigo.

The R protein has no clinical or pathogenic importance.

CELL WALL CARBOHYDRATE. The streptococci have been divided into groups A through O according to differences in the carbohydrate layer of the cell wall. In groups A and C the carbohydrate layer is made up of polymers of *N*-acetylglucosamine and rhamnose and is referred to as the C substance. Many investigators believe that the pathogenesis of rheumatic fever is related to the C substance since group A carbohydrate cross-reacts with the glycoprotein of heart valves and joints. Cell wall components containing the C substance can cause severe arthritis when injected into rabbits. In addition patients with rheumatic fever have a high level of serum antibodies to group A carbohydrate.

CELL WALL PEPTIDOGLYCAN. The peptidoglycan of the cell wall of streptococci consists of repeating units of *N*-acetylglucosamine and *N*-acetylmuramic acid cross-linked by peptide bridges. This layer has been shown to be antigenic and is capable of producing fever, dermal necrosis, carditis, and lysis of red blood cells in rabbits and other animals. Its toxicity is similar to that of the endotoxins of gram-negative bacteria. The biosynthesis of peptidoglycan by *S. pyogenes* is also the target for the various penicillins.

CYTOPLASMIC MEMBRANE. Antigenic components in the cell membrane cross-react with heart, kidney, and connective tissue. It has been suggested that these streptococcal components can interact with host tissue and cause severe damage. As with many other streptococcal antigens, no direct relationship has ever been established between the membrane antigen and the disease condition. The group D–specific antigen is a component of the cell membrane and not of the cell wall, as is the case with all other groups of streptococci.

Extracellular Streptococcal Products

The streptococci elaborate extracellular products in vivo and in vitro. Many of these products are believed to be important in the pathogenesis of streptococcal infections. They are used in diagnosing such infections.

ERYTHROGENIC TOXIN. In some upper respiratory infections the infecting streptococcal strain produces an extracellular toxin called *erythrogenic toxin*. Only lysogenic strains can elaborate this toxin. In the nonimmune individual it causes a rash that is characteristic of *scarlet fever*. The toxin alone is not lethal, and its role in the rash that characterizes scarlet fever is not known. The response of humans to intradermal injection of the toxin is the basis of the *Dick test*, which is seldom performed today in the United States. In a positive test, a localized erythematous reaction occurs at the site of injection, indicating that the patient has no circulating antitoxin in his or her serum. If antitoxin is injected intradermally, the rash disappears. This reaction, called the *Schultz-Charlton* reaction, is used diagnostically to identify the scarlet fever rash. In a negative Dick test the toxin is neutralized by circulating antitoxin, and there is no inflammatory response to the toxin.

CARDIOHEPATIC TOXIN AND NEPHROTOXIN. A low molecular weight compound excreted by virulent strains of streptococci is capable of producing lesions in the heart and liver tissue when injected into susceptible laboratory animals. The exact nature or function of this cardiohepatic exotoxin in human infection is not clear. It is believed that by damaging tissue the toxin prepares the way for streptococcal colonization.

Acute glomerulonephritis is a complication that

follows primary streptococcal infection. The disease in humans resembles the nephritis that can be induced in animals by the injection of streptococcal products. The disease is believed to be the result of an immune complex consisting of streptococcal antigens and antibodies, but this has not been confirmed. Some studies have indicated that a nephrotoxin released by the infecting streptococci may be responsible for the lesions.

HEMOLYSINS. The hemolytic activity of many streptococcal strains is due to the production of two distinct extracellular hemolysins called *streptolysin O (SLO)* and *streptolysin S (SLS)*. SLO is oxygen labile, and its activity is dependent on reducing conditions. It is immunogenic, and detection of anti–streptolysin O antibodies is extensively used in the diagnosis of streptococcal infections. When SLO is injected into susceptible laboratory animals, it can lyse red blood cells and leukocytes and is especially toxic to cardiac tissue. SLO has been implicated as a factor in the pathogenesis of rheumatic fever, a poststreptococcal complication.

SLS is oxygen stable and is responsible for the zone of beta hemolysis seen around surface colonies of streptococci on blood agar plates. Like SLO, SLS is capable of lysing mammalian red blood cells and leukocytes. Unlike SLO, SLS is nonimmunogenic. Intravenous injections of SLS in rabbits cause intravascular hemolysis and acute liver necrosis.

SPREADING FACTORS. In primary streptococcal infections the infecting strain is often capable of spreading to surrounding tissue. This capability is probably due to the formation of extracellular products that can dissolve the matrix of connective tissue and clotted material accumulated as a result of host inflammatory responses. The spreading factors include the following:

1. Hyaluronidase. Hyaluronic acid, the substrate for the enzyme hyaluronidase, is a component of intercellular substance. Depolymerization could therefore enhance the spread of streptococci in the tissue.
2. Proteinase. A proteolytic enzyme released by many group A streptococci can digest some peptides and proteins. This streptococcal proteinase can split chondroitin sulfate and hyaluronic acid of connective tissue. The purified proteinase when injected into susceptible animals has been shown to cause extensive lesions of the heart.
3. Streptokinase. Streptokinase is produced by most group A streptococci. It can interact with the proenzyme plasminogen of human serum, converting it to plasmin. Plasmin can digest fibrin and other serum factors important in the formation of blood clots.
4. Nucleases. At the site of infection the host's inflammatory response results in the accumulation of nuclear exudate from dead or injured white blood cells. Virulent streptococci can produce nucleases such as ribonuclease and deoxyribonuclease that will digest the DNA or RNA of the exudate, thus facilitating the spread of trapped streptococci. The released purine and pyrimidine bases can also be used nutritionally by the streptococci.

Pathogenesis

As stated previously, more than 90 percent of streptococcal diseases are caused by group A beta-hemolytic streptococci. Streptococcal disease is divided into two categories: the suppurative, or primary infections, and the nonsuppurative, or those regarded as complications following primary streptococcal infections.

SUPPURATIVE DISEASES. Suppurative diseases are those in which pus is formed. One group of suppurative streptococcal diseases often begins as acute pharyngitis, which can be complicated by meningitis, otitis media, and pneumonia. The other group includes puerperal fever, as well as such skin infections as impetigo, cellulitis, and erysipelas.

Impetigo is a highly contagious skin disease found primarily in children. The infection begins as small blisters that can spread to adjacent areas. The sores become covered with crusts. If the infecting strain is nephritogenic, a streptococcal complica-

tion called acute glomerulonephritis, mentioned earlier, can result.

Cellulitis is an inflammatory condition associated with streptococcal invasion of connective tissue. This type of disease can occasionally result in gangrene with subsequent invasion of the bloodstream. *Puerperal fever* is a uterine infection that frequently accompanies childbirth when aseptic techniques are not followed. *Erysipelas* is an infection of the skin or mucous membrane and is characterized by a spreading inflammation. It is frequently seen on the face.

In suppurative infections the virulence of the streptococcus is directly related to the presence of M protein, the antiphagocytic factor. Laboratory experiments have clearly demonstrated that when virulent strains have their M protein removed by trypsin, they are easily phagocytized. In the majority of primary streptococcal infections the microorganisms are destroyed by the host's immune system. In some instances, however, because of either the particular streptococcal strain involved or the host's immunological state, nonsuppurative complications develop.

NONSUPPURATIVE DISEASES. The nonsuppurative complications that follow primary streptococcal infections are scarlet fever, rheumatic fever, acute glomerulonephritis, and erythema nodosum. Their exact cause is still in doubt. One theory holds that a toxin or toxic factor directly affects the tissue; another maintains that it is the host's response to the microbe or its products that damages the tissue.

Scarlet fever is associated with the formation of erythrogenic toxin by group A streptococci and has already been discussed.

Rheumatic fever is a condition that follows infection by any number of group A streptococcal types. It is characterized by heart muscle damage and is often accompanied by tissue destruction of heart valves as well as inflammation of the joints. The highest incidence of rheumatic fever is among those aged 5–19 years, especially among the urban poor of all races and ethnic groups.

Many authorities consider rheumatic fever an autoimmune disease. This theory is related to the finding that many antigenic components of the streptococcal cell wall can cross-react with cardiac tissue. For example, there is a cross reactivity between the group A polysaccharide of the cell wall and heart valve glycoprotein. In rheumatic fever, if there is heart valve damage, group A polysaccharide antibodies are at a high level. If the damaged valves are manipulated during surgery, the level of antibodies increases. If the damaged valves are removed, the antibody level decreases. In addition, antigens are present in the cytoplasmic membrane of the group A streptococcus that are related to antigens in the heart muscle. There is a sustained rise in the titer of antibodies to streptococcal membrane that cross react with cardiac tissue preceding an attack of rheumatic fever. This suggests, but does not prove, that antibodies may play a role in the pathogenesis of rheumatic fever.

None of the antibodies described above have been found to be toxic to heart tissue and thus the pathogenesis of rheumatic fever may be caused by factors other than or in addition to immunologic factors. Studies in animals indicate that streptococcal extracellular toxins or cellular components may be directly toxic to heart tissue. Streptolysin S or O, for example, have been shown to be toxic to cellular membranes. Streptococcal cell wall components have been shown to persist in tissues such as skin, heart, and liver for long periods of time. The length of time in the tissue correlates with the duration of the disease. These cell wall components are presumably present in the lesion because of the poor hydrolytic activity of phagocytic enzymes on the streptococcal cell wall. When streptococcal fragments containing the C substance are injected into mice, they bring about heart lesions similar to those seen in rheumatic fever. Injections of whole cells, however, have no effect unless the heart tissue has already been damaged. The peptidoglycan of the cell wall is also toxic to mammalian tissue. When injected intradermally into rabbits, the peptidoglycan produces lesions similar to those seen in erythema nodosum in humans.

Acute glomerulonephritis (AGN) is a nonsuppurative complication that follows throat or skin infections by a relatively few serological types of group A streptococci. Clinical symptoms, which appear 1–4 weeks after the primary infection, include pro-

teinuria, hematuria, and oliguria, but no bacteria can be recovered. The pathogenesis of AGN is believed to be due to an immune complex reaction. Antigens from the cytoplasmic membrane of the bacterial cell are believed to cross-react with the basement membrane of the glomerulus of the kidney, precipitating the immune complex reaction. Antibiotics are of no use in the treatment of AGN; only bed rest and administration of salicylates are of therapeutic value.

Erythema nodosum is a skin condition in which small red nodules appear under the surface of the skin. It exists in a variety of other diseases such as tuberculosis and coccidioidomycosis. Many authorities believe that erythema nodosum is the result of hypersensitivity to the peptidoglycan portion of the streptococcal cell wall.

Laboratory Diagnosis

The clinical symptoms of many streptococcal infections are shared by other bacterial and viral diseases. The clinical symptoms of acute pharyngitis, for example, are similar to those of numerous respiratory virus infections. In a certain percentage of patients with streptococcal disease the "typical" symptoms or host immunological responses do not appear. Accurate diagnosis of most streptococcal infections requires, therefore, both clinical and bacteriological support. In preliminary identification procedures the majority of laboratories rely on the hemolytic behavior, bacitracin sensitivity, and fluorescent antibody response of isolated streptococcal species. Presumptive identification of group A beta-hemolytic streptococci in patients with pharyngitis can be made by swabbing the throat and streaking on sheep blood agar, and adding a bacitracin sensitivity disk. Group A streptococci are sensitive to bacitracin. Recently a kit (Phadebact) has been developed that detects the major beta-hemolytic groups (A, B, C, and G) with more reliability than does the bacitracin sensitivity test. The test is based on a coagglutination principle in which group-specific IgG antistreptococcal antibodies are coupled to dead "carrier" staphylococci. The reagent is mixed with the unknown streptococcal growth, and a specific reaction is indicated by agglutination. The test can be performed in 17 hours, compared with the usual 48–72 hours required for determining biochemical or serological properties. A second coagglutination test called Streptex is also available. In this test, latex particles are conjugated to group-specific streptococcal antisera.

Group A streptococcal antigen can also be detected directly from throat swabs. A nitrous acid extraction procedure in combination with coagglutination can yield results in 30 minutes. However, large numbers of streptococci are required on the swab.

The anti–streptolysin O (ASO) titer of the serum remains the principal diagnostic test for poststreptococcal disease. Often the test is inconsistent, however; for example, about 20 percent of rheumatic fever patients have normal serum, while impetigo patients demonstrate a variable ASO titer.

Diagnostic problems can be overcome by measuring antibodies to other streptococcal products such as hyaluronidase (HA), deoxyribonuclease (DNase), nicotinamide-adenine dinucleotidase (NADase), and streptokinase (SK). Because some of the reagents for these tests are expensive and the tests are time-consuming, laboratories are seldom equipped to measure these streptococcal antibodies. A 2-minute slide agglutination test called the Streptozyme test has been developed. The reagent is sheep red blood cells that have been sensitized with the extracellular products (i.e., HA, DNase, NADase, and SK) from group A streptococci. The test specifically measures anti-HA, anti-DNase, anti-NADase, and anti-SK antibodies. Diluted patient serum is mixed with the sensitized sheep red blood cells on a glass slide, and agglutination is noted. The test is an excellent screening device and has detected rheumatic fever in patients who were serologically negative in the ASO test.

The biochemical tests used to separate various streptococcal groups are compared in Table 19-3.

Treatment and Prevention

To date, all strains of group A beta-hemolytic streptococci are susceptible to penicillin. Penicillin is therefore the drug of choice in infections caused by this group. Penicillin G benzathine is usually prescribed because of its prolonged action. Follow-up

Table 19-3. Tests Used in the Presumptive Identification of Streptococci

Test	Group A	Group B	Group D	Viridans	Pneumococcus
Susceptibility to bacitracin	+	−	−	+[a]	±
CAMP reaction[b]	−	+	−[a]	−[a]	−
Hippurate hydrolysis	−	+	−	−[a]	−
Bile esculin reaction	−	−	+	−[a]	−
Growth in 6.5% NaCl	−	+[a]	−[c]	−	−
Optochin susceptibility and bile solubility	−	−	−	−	+

[a]Occasional exceptions.
[b]For an explanation of the CAMP reaction, see under Group B Streptococci.
[c]Only group D enterococci.
+ = positive or susceptible; − = negative or resistant.

cultures should be taken after discontinuation of therapy to determine whether all streptococci have been eliminated. The prevention of streptococcal infections also prevents rheumatic fever. Penicillin treatment does not alter the course of rheumatic fever. To minimize the risk of streptococcal infections in rheumatic fever patients, an injection of penicillin G benzathine is usually prescribed each month. This regimen is considered necessary for at least 5 years after the rheumatic attack. For individuals sensitive to penicillin, erythromycin is a suitable alternative. Vaccines prepared from streptococcal type-specific M protein have been used successfully as immunizing agents against group A infections. Because of the many different streptococcal types, a vaccine utilizing all the type-specific M proteins has proved impractical.

GROUP B STREPTOCOCCI

S. agalactiae, the species designation for members of group B streptococci (GBS), has become an important pathogen. Group B streptococci are an important cause of neonatal disease and are the most frequent cause of life-threatening disease in newborns in the United States.

Group B streptococci are found in the oral cavity, intestinal tract, and vagina. Neonatal infection by GBS occurs primarily during passage through the birth canal. Factors conducive to GBS infection are premature labor, prolonged rupture of placental membranes, and birth weight less than 3 pounds. The incidence of GBS infection in the United States is 1.1 to 3.7 per 1000 live births; the case fatality ratio is 22 to 80 percent.

There are two forms of GBS disease in neonates, early onset and late onset. Early onset infection occurs within the first few days of life, develops rapidly, and has a high mortality rate. This is the most common form of the disease and is frequently associated with sepsis or pneumonia. Late onset disease occurs insidiously and is difficult to distinguish from other bacterial infections. Meningitis is the most frequent complication of late onset infection.

Group B streptococci can be divided into five distinct serotypes. One of these, type III, is the most common cause of neonatal infection. Experimental studies with type III have demonstrated that a capsular polysaccharide component, sialic acid, appears to be an important virulence determinant. Sialic acid has been shown to inhibit the alternative complement pathway; thus resistance to disease requires specific antibodies.

Group B streptococcal infections can be treated with penicillin; however, GBS are not as susceptible to penicillin as are group A streptococci. Ampicillin or ampicillin plus gentamicin has been shown to be effective in treatment. Prevention of disease has been approached in two ways. Intravenous intrapartum therapy with ampicillin has been shown

to prevent neonatal colonization with GBS. A second technique is to treat the neonate with penicillin less than 2 hours after birth. Prophylaxis is recommended when the factors conducive to group B streptococcal infection (listed earlier) are encountered.

Group B strains are easily distinguished from other streptococci by their ability to hydrolyze hippurate and their resistance to the antibiotic bacitracin. The CAMP test is also used as a presumptive test for GBS identification. The CAMP test, named from the initials of the investigators who perfected it, is based on the production of a "CAMP factor" elaborated by group B streptococci. The test is performed by making a single streak of a streptococcal strain perpendicular to but not touching a disk containing betalysin on a sheep blood agar plate. If the streptococcal strain produces the CAMP factor a crescent-shaped clearing appears at the juncture of the betalysin-containing disk and the streptococci. The CAMP factor thus enlarges the zone of lysis produced by betalysin.

GROUP C STREPTOCOCCI

Group C streptococci are a frequent cause of infection in many animal species but are a rare cause of infection in humans. There are four species of group C streptococci. *S. equisimilis* is primarily associated with human illness in the United States. Human illnesses include endocarditis, pneumonia, meningitis, epiglottitis, and wound infections. Outbreaks of disease have been associated with unpasteurized milk or cheese made from unpasteurized milk. *S. zooepidemicus*, a group C streptococcus, has been associated with outbreaks of pharyngitis and nephritis in Europe and recently with illness in the United States. (See boxed essay, p. 464.)

ALPHA-HEMOLYTIC STREPTOCOCCI

The alpha-hemolytic streptococci include important species found in the oral cavity: *S. mitis* (also called *S. mitior*), *S. salivarius*, *S. sanguis*, and *S. mutans*. These are not primary pathogens but act as opportunistic challengers to the host. Many of the alpha-hemolytic species play a role in dental caries (discussed in Chapter 37). Some enterococci (group D) may also be alpha hemolytic.

Species of alpha-hemolytic as well as nonhemolytic streptococci have cell wall polysaccharides that cross-react with the capsular material of the pneumococci. The close genetic relationship between them has been demonstrated in transformation experiments. In such experiments the extracted DNA of encapsulated streptococci is able to transform nonencapsulated pneumococci to capsular types.

The major disease associated with the alpha-hemolytic streptococci is subacute bacterial endocarditis (SBE). SBE, a suppurative disease, is generally preceded by dental extraction or some type of oral surgery that displaces the alpha-hemolytic species from the oral cavity into the bloodstream. The disease occurs most frequently in patients with rheumatic fever or congenital heart disease. The dislodged streptococci are capable of localizing on the affected heart tissue and can bring about further heart valve damage. Left untreated, SBE can lead to heart failure, but the disease can be treated with antibiotics. The American Heart Association has recommended the following prophylactic regimen:

1. For those with congenital heart disease or rheumatic fever, penicillin G plus penicillin G procaine is given intramuscularly 30 minutes to 1 hour before oral surgery (some physicians prefer erythromycin to penicillin because rheumatic fever patients already use penicillin to prevent recurrences of the disease). Penicillin V (phenoxymethyl penicillin) is taken orally every 6 hours for 48 hours after surgery. Vancomycin may be substituted if the patient is allergic to penicillin.
2. For those with prosthetic valves, regimen 1 may be used, or penicillin G plus penicillin G procaine plus streptomycin is administered intramuscularly 30 minutes to 1 hour before oral surgery. Penicillin V is taken orally every 6 hours for 48 hours after oral surgery.

GROUP C STREPTOCOCCAL INFECTIONS ASSOCIATED WITH EATING HOMEMADE CHEESE—NEW MEXICO

Between July 25 and September 9, 1983, 16 cases of invasive group C streptococcal infection were identified in northern New Mexico. The group C streptococci were isolated from the blood of 15 patients and the pericardial fluid of one patient. The organism isolated from 14 of the patients has been identified as a group C beta-hemolytic streptococcus, species *S. zooepidemicus*; the species of the remaining two isolates have not yet been determined. Ages of the 16 patients ranged from 19 to 89 years. All were Hispanic.

In general the clinical syndrome was characterized by fever, chills, and vague constitutional symptoms. However, five patients had localized signs of infection, including pneumonia, endocarditis and meningitis, pericarditis, and abdominal pain that led to a cholecystectomy for one patient and an appendectomy for another patient. Two patients with multiple underlying medical problems died.

Initial questionnaires identified eating *queso blanco*, a homemade white cheese, as the only risk factor associated with illness (10 of 15 case patients vs. 7 of 45 controls). During subsequent discussions four of the five patients who did not report in the case-control study that they had eaten the homemade cheese later remembered that they had consumed the cheese before the onset of illness. Thus, only one patient did not recall having eaten the cheese before becoming ill.

The sole source of the homemade cheese consumed by the patients was an ungraded, small (seven cows), family dairy farm in northern New Mexico. At the farm, the cheese was made from raw cow's milk and was not subsequently aged. It was distributed to several stores in northern New Mexico within 24–48 hours after preparation in the family kitchen. Milk samples from the cows and cheese samples from the stores were obtained for microbiological analysis. Group C beta-hemolytic streptococci, species *S. zooepidemicus*, have been isolated from multiple samples of each.

Public health control measures included closing the dairy operation, removing the cheese from the stores, and advising the public to dispose of any *queso blanco* purchased from the stores that sold the implicated product. No new cases of group C streptococcal infections have been identified since these interventions were implemented.

From *MMWR* 32:510, 1983.

GROUP D STREPTOCOCCI

Most group D streptococci are either alpha hemolytic or nonhemolytic on sheep blood agar. Although most species are found in the intestinal tract of humans and animals, some have also been found in the oral cavity. As stated earlier, the group D antigen is located in the cytoplasmic membrane and not the cell wall.

The species included in the group are *S. faecalis, S. faecium, S. durans, S. bovis*, and *S. equinus*. *S. faecalis* and *S. faecium* are of intestinal origin and are referred to as enterococci. Medically, the major species of group D streptococci is *S. faecalis*. *S. faecalis* has been implicated in urinary tract infections and abdominal lesions, particularly in patients compromised by bowel surgery. *S. faecalis* may also cause SBE. Group D streptococci are resistant to a number of antibiotics. Penicillin plus streptomycin is often the drug therapy of choice in group D infections.

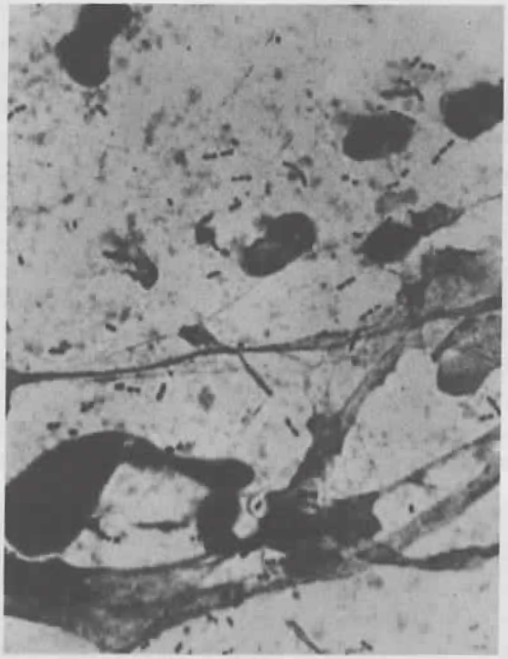
ANAEROBIC STREPTOCOCCI

The anaerobic streptococci are classified in the genera *Peptococcus* and *Peptostreptococcus*. Most are nonhemolytic. They have been incriminated in such infections as puerperal sepsis, SBE, and deep wound abscesses. This group of streptococci is found in the intestinal tract and the vagina. Many strains are resistant to penicillin but sensitive to bacitracin and chloramphenicol. Discussion of the importance of these cocci in anaerobic infections is continued in Chapter 39.

STREPTOCOCCUS PNEUMONIAE (PNEUMOCOCCI)

Until recently the pneumococci were treated as a distinct genus: *Diplococcus*. Now the eighth edition of *Bergey's Manual of Determinative Bacteriology* has placed the pneumococci in the genus *Streptococcus*. The genetic relationship between the two genera was previously described in the discussion of alpha-hemolytic streptococci: Pneumococcal cells are capable of being transformed by streptococcal DNA, and this characteristic has been important in genetic studies.

Fig. 19-9. Sputum sample taken from patient with pneumococcal pneumonia. The pneumococci appear lancet shaped in pairs or short chains. (From A. S. Klainer and I. Geis, *Agents of Bacterial Disease*. New York: Harper & Row, 1973.)



General Characteristics

The pneumococci are inhabitants of the upper respiratory tract and, depending on seasonal variations, can be found in 30 to 70 percent of the population. They are gram-positive cocci that occur singly, in pairs (diplococci), or in chains. The typical paired or short-chain forms appearing primarily in clinical specimens are often lancet shaped and surrounded by a capsule (Fig. 19-9). Only encapsulated (smooth form) strains are virulent. On repeated subculture in the laboratory the capsule is lost (rough form). There are approximately 83

pneumococcal serotypes, differing in the composition of their capsular polysaccharide.

The pneumococci are facultative anaerobes that are very fastidious in their cultural requirements. Some strains need an elevated level of CO_2 for initial isolation. Meat extracts supplemented with defibrinated blood are one of the best cultural media for isolation of pneumococci. The addition of blood to culture media supplies the enzyme catalase, which is not produced by the pneumococci. Under aerobic conditions the pneumococci produce hydrogen peroxide, which can be toxic to the pneumococci. The enzyme catalase acts to remove the accumulated hydrogen peroxide. On blood agar pneumococcal colonies exhibit alpha hemolysis and closely resemble colonies of alpha-hemolytic streptococci.

Lobar pneumonia is a common and serious form of pneumococcal infection. The pneumococci may also be involved primarily or secondarily in such complications as meningitis, otitis media, bronchitis, and peritonitis.

Pathogenesis

The primary virulence factor of the pneumococci is the capsular polysaccharide. The capsule prevents binding of antibody to the cell wall of the pneumococcus and thus inhibits phagocytosis. Pneumococci also produce a hemolysin (pneumolysin) and neuraminidase. Their role in pathogenicity is not known.

As stated earlier, as much as 70 percent of the population at any one time may carry pathogenic pneumococci in the upper respiratory tract. Many authorities believe that pneumonia and related pneumococcal infections are acquired endogenously through lowered host resistance rather than exogenously by direct contact. Age, underlying illness, and viral infections of the upper respiratory tract are important predisposing factors to pneumococcal infections. In the healthy individual numerous barriers prevent establishment of infection in the lungs and bronchioles. Mucus-laden pneumococci can be expelled naturally by the combined action of ciliated epithelium and the cough reflex or through the phagocytic action of macrophages lining the alveoli of the lungs. If the physical and immunological barriers of the host are impaired—as, for example, in the debilitated person or chronic alcoholic—the pneumococci can infect the respiratory tree. The encapsulated pneumococci become trapped in the bronchioles, where they proliferate and eventually infect the alveoli. The inflammatory response of the host leads to the accumulation of an alveolar exudate consisting of large numbers of neutrophils and red blood cells. During this stage the patient's sputum also resembles the alveolar exudate and will appear bloody. During the recovery stage the pneumococci are phagocytized, the alveolar exudate is resorbed, and the lung tissue appears as healthy as before infection. When immediate recovery does not take place, the alveoli can become fibrous and inelastic because of deposition of fibroblasts, and breathing becomes difficult.

Complications of pneumococcal infection include bronchitis, otitis media, mastoiditis, and meningitis. In patients with underlying illnesses, such as sickle cell anemia, pneumonia often leads to bacteremia and meningitis. Bacteremia is present in 25 percent of the adult population exhibiting pneumococcal disease.

In the inflammatory stage of pneumonia, as well as with other diseases in which there is an inflammatory process, a special protein called the *C-reactive protein* is found in the blood (see Chapter 14). It precipitates a polysaccharide component of the pneumococcal cell wall called the C substance (discussed earlier in this chapter).

Laboratory Diagnosis

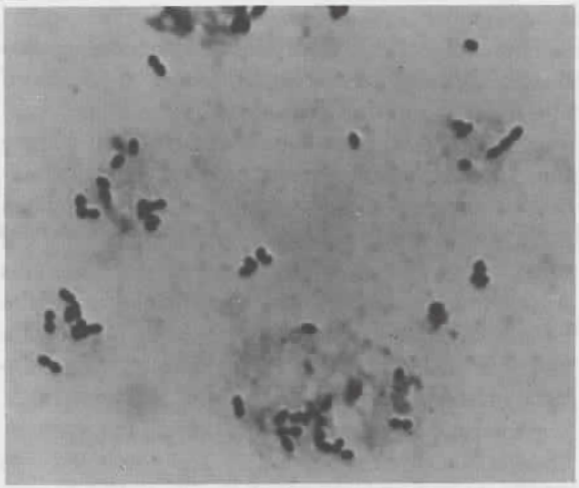
Presumptive identification of pneumococci can be made by Gram-staining sputum cultures. This procedure is complicated by the fact that, microscopically, pneumococci resemble alpha-hemolytic streptococci, which are often found as contaminating species in clinical specimens. A more rapid and accurate identification procedure involves applying an anticapsular serum to the sputum samples and observing capsular swelling (called the *quellung* reaction). If sputum samples cannot be obtained for examination, swabs of the laryngeal area or material aspirated from involved areas of the lung

should be examined. Transtracheal aspiration is also widely used for obtaining samples for direct examination.

A definitive diagnosis of pneumococcal pneumonia can be made only if the microorganisms are isolated directly from the blood or from other clinical specimens by plating onto blood agar. A useful adjunct to direct isolation is identification of specific pneumococcal antibodies by counterimmunoelectrophoresis of body fluids—pleural fluid, blood, urine. Once cultured, the pneumococci can be differentiated from alpha-hemolytic streptococci by the following procedures:

1. Quellung reaction. Provided antiserum is available, the quellung reaction is the most accurate and specific test for identification of the pneumococci (Fig. 19-10). Emulsified sputum or exudative material is spread on a slide and mixed with antiserum against type-specific polysaccharide or the polyvalent antiserum, which contains antibodies to 33 of the most prevalent types in the United States or all 83 pneumococcal types.
2. Optochin susceptibility test. Optochin is an antimicrobial drug derived from quinine. When disks containing the drug are placed on blood agar previously seeded with pneumococci, zones of inhibition can be observed around the optochin. Alpha-hemolytic streptococci are resistant to optochin.
3. Bile solubility test. The pneumococci produce an enzyme (amidase) that cleaves specific covalent bonds in the peptidoglycan layer. This enzyme is activated by bile or bile salts such as sodium deoxycholate. When a few drops of 10% sodium deoxycholate solution are added to a broth culture of pneumococci, the cells are rapidly lysed. Alpha-hemolytic streptococci are not lysed by bile.
4. Mouse virulence test. Most pneumococcal strains, when injected intraperitoneally into mice, can induce death of the animals within 24 to 48 hours. Alpha-hemolytic streptococci, used under the same procedures, are not lethal to mice.

Fig. 19-10. Quellung reaction. A. Control (absence of antiserum). B. Capsular swelling of bacteria after application of antiserum. A capsular halo can be observed around the diplococci. (Courtesy R. Austrian.)



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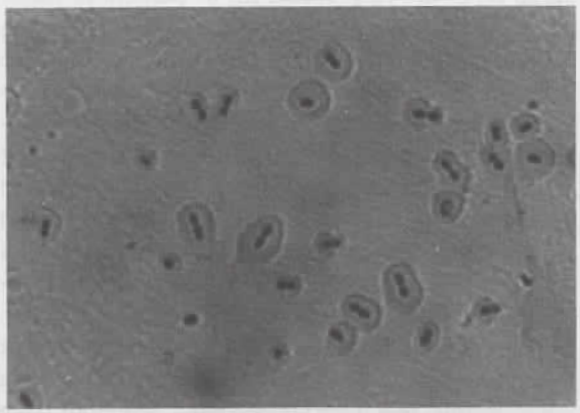

B

Treatment and Prevention

Penicillin is the drug of choice in the treatment of pneumococcal infections. Very few strains become resistant to penicillin. In patients harboring penicillin-resistant strains or those who are sensitive to the drug, erythromycin or tetracycline is an effective substitute. Of major concern is the appearance of two new strains of *S. pneumoniae* in South Af-

rica. They are resistant not only to penicillin but to most of the commonly used antibiotics, that is, chloramphenicol, tetracycline, erythromycin, and clindamycin. They are sensitive only to drugs that are highly toxic; indeed, use of those drugs leads to a rapid development of resistance.

The mortality is high in pneumococcal pneumonia, especially in the high-risk groups—the aged and those compromised by underlying illnesses. A vaccine could save the lives of these patients. Since there are 83 different pneumococcal types, a universal vaccine is somewhat impractical. It has been proposed that a vaccine contain only the virulent types most frequently involved in disease. This would require a continued surveillance as to the pneumococcal types usually implicated in infection and their geographical location. A vaccine containing polysaccharide antigens from 23 types of *S. pneumoniae* was developed in 1983. Each polysaccharide is extracted separately and combined into the final product (polyvalent vaccine). The 23 bacterial types are responsible for 87 percent of pneumococcal disease in the United States. The polyvalent vaccine causes a twofold or greater increase in type-specific antibody within 2–3 weeks after vaccination. The vaccine is less antigenic for children under 2 years of age. It is recommended for adults with chronic disease and in epidemic-prone areas such as military bases. The vaccine is also recommended for children over 2 years of age with chronic illness specifically associated with increased risk for pneumococcal disease. The adverse reactions from vaccination are very mild but can be severe if the vaccine is given more than once. Arthus reactions and systemic reactions have been reported when "booster doses" of vaccine were administered.

SUMMARY

There are about ten species of *Staphylococcus* that infect or colonize humans. The only regularly pathogenic species, *S. aureus*, is the only species that is coagulase positive. The other species collectively are referred to as the CONS—the coagulase-negative staphylococci.

S. aureus is specifically or primarily associated with various lesions of the skin and associated structures and with osteomyelitis, toxic shock syndrome (TSS), scalded skin syndrome (SSS), and staphylococcal food poisoning and enteritis.

There is a relatively high and constant carrier rate of *S. aureus*. Hospital personnel have an even higher carrier rate than the general population, and that poses a problem for debilitated hospitalized patients since they are prone to staphylococcal infections. Carriers can be traced by phage typing.

Mainstays of laboratory identification are the Gram-stained smear and the coagulase test. Penicillinase-producing strains and strains that exhibit tolerance complicate chemotherapy for staphylococcal infections.

CONS, especially *S. epidermidis*, are increasingly recognized as opportunistic pathogens in patients with wounds and those with prostheses. *S. saprophyticus* has a predilection to infect the urinary tract, especially in young women.

Streptococci are gram-positive spherical cells that occur in chains of varying length. The major pathogens are differentiated into various groups based on the cell wall carbohydrate. Most disease is caused by group A streptococci (*S. pyogenes*), but other groups such as B, C, D, and G are also known to cause disease. The streptococcal surface consists of several antigens that are associated with virulence. In addition the streptococci elaborate several products that are believed to be associated with pathogenesis. These products and antigens are helpful in the classification and differentiation of the streptococci. Group A streptococci are involved in two types of disease: suppurative (impetigo, puerperal fever, and erysipelas) and nonsuppurative (scarlet fever, rheumatic fever, acute glomerulonephritis, and erythema nodosum). Rheumatic fever and acute glomerulonephritis are complications of streptococcal infection. Group B streptococci (*S. agalactiae*) are important agents of neonatal disease. Group D streptococci cause disease in compromised patients. Diagnosis of strep-

tococcal disease is based on clinical signs, hemolytic behavior, fluorescent antibody results, and biochemical tests.

S. pneumoniae is a normal inhabitant of the upper respiratory tract. The organism's virulence is directly associated with the presence of an antiphagocytic capsule. The organism is a leading cause of pneumonia in the very young and in chronically ill older adults. The organism can be detected in clinical specimens by application of antiserum to the specimen and observation of capsular swelling (quellung reaction). *S. pneumoniae* as well as other streptococci are very sensitive to penicillin. A pneumococcal vaccine containing the antigens from the most virulent strains has been developed and is currently available for elderly persons with chronic ailments and others at risk of pneumococcal disease.

QUESTIONS FOR STUDY

1. Differentiate among the types of skin lesions caused by *S. aureus*.
2. Give the full names of the syndromes that are represented by the letters *TSS* and *SSS*. Describe the syndromes, and name the virulence factors that specifically are associated with the syndromes.
3. Explain the selective and the differential features of mannitol salt agar.
4. How is *S. aureus* differentiated from *S. epidermidis*?
5. Describe two ways that staphylococcal coagulase is important.
6. What is meant by staphylococcal tolerance?
7. Explain how phage typing is used as an epidemiological tool.
8. Prepare a sketch to explain the coagglutination test.
9. What is meant by the CONS? Why is there an increasing medical interest in this category of staphylococci?
10. What is the most important species of *Streptococcus* that is pathogenic for humans?
11. What are the techniques for separating the various pathogenic streptococci?
12. What is the major virulence factor for *S. pyogenes*? *S. pneumoniae*?
13. What are believed to be the reasons for destruction of cardiac tissue in rheumatic fever?
14. What is the importance of group B streptococci? Which group of humans is most adversely affected by these pathogens?
15. How is *S. pneumoniae* detected in the laboratory?
16. What biochemical tests differentiate *S. pneumoniae* from the alpha-hemolytic streptococci?
17. The alpha-hemolytic streptococci are often associated with what disease or diseases?

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20. THE GRAM-NEGATIVE DIPLOCOCCI, THE *NEISSERIA*, AND RELATED GENERA

OBJECTIVES

To describe the properties of the *Neisseria* that distinguish them from other genera of bacteria

To describe the properties that are used to differentiate the species within the genus *Neisseria* and the properties that differentiate the pathogens from the nonpathogens

To describe *Neisseria meningitidis* in terms of the diseases it causes, epidemiology, antigens, laboratory diagnostic procedures, chemotherapy, and active immunization

To describe the prominent clinical aspects of genital and extragenital gonorrheal infections and the laboratory diagnostic procedures, chemotherapy, virulence factors, and prospects for a vaccine

To name other members of the Neisseriaceae family that are opportunistically pathogenic

General Characteristics of *Neisseria*
Differentiation Among Species of *Neisseria*
Properties of the *Neisseria* Pathogens: *N. meningitidis* and *N. Gonorrhoeae*
- Fragility
- Cultural Fastidiousness
- Requirement for Elevated CO_2 Levels
- Intracellular Habitat in Acute Infections
- *Neisseria meningitidis*—the meningococcus
  - Meningococcal Disease
    - Nasopharyngitis
    - Meningococcal septicemia
    - Meningococcal meningitis
  - Epidemiology
  - Antigenic Composition
  - Laboratory Identification
    - Isolation, Carbohydrate Utilization Tests, Oxidase Test
      - Cytochrome oxidase test
    - Laboratory Diagnosis of Meningococcal Meningitis
      - Serological tests performed on cerebrospinal fluid
  - Prevention and Control
    - Chemotherapy
    - Active Immunization
    - Chemoprophylaxis
- *Neisseria gonorrhoeae*—the gonococcus
  - Gonococcal Diseases
    - Genitourinary Tract Infections in the Male
    - Genitourinary Tract Infections in the Female
      - Pelvic Inflammatory Disease, Upper Tract Disease
    - Extragenital Infections
      - Local Infections
      - Disseminated Gonococcal Infection
  - Epidemiology
  - Virulence Factors
    - Pili
    - IgA Protease
    - Capsule
    - Epithelial Endocytosis
    - Endotoxins
  - Laboratory Diagnosis
    - Examination of Stained Smears of Clinical Material
    - Identification Procedures Conducted on Isolates
      - Colony Type
      - Carbohydrate Metabolism Reactions
      - Superoxol (Catalase) Test
      - Serological Tests
      - Limulus Lysate Test as an Exclusion Test for Cervical Gonorrhea
  - Chemotherapy
    - New Developments
    - Chemotherapy for Uncomplicated Gonococcal Infections
  - Prophylaxis of Ophthalmia Neonatorum
  - Immunity
    - Immune System Response
    - Failure to Develop a Solid Immunity
    - Prospects for a Vaccine

Other Members of the Family *Neisseriaceae*
- Nonpathogenic *Neisseria* Species
- Acinetobacter
- Branhamella
- Moraxella

The medically important members of the genus *Neisseria* are *N. meningitidis* (the meningococcus) and *N. gonorrhoeae* (the gonococcus). The other members included in Table 20-1 are nonpathogens that colonize the oral cavity and the upper respiratory tract.

GENERAL CHARACTERISTICS OF *NEISSERIA*

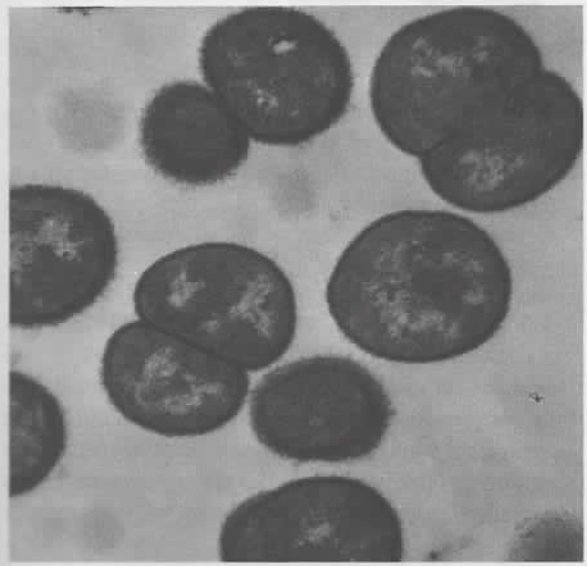
1. All species are gram-negative diplococci. Where the members of the paired bacteria abut, they are flat sided, so that each member of the pair presents a bean-shaped configuration (Fig. 20-1).

Table 20-1. Characteristics of the Neisseriae

Species	Acid formed from the degradation of					Production of IgA protease	Production of pigment	Growth on nutrient agar at 35°C	Growth on blood agar at 22°C
	Glucose	Maltose	Sucrose	Fructose	Lactose				
N. gonorrhoeae	+	−	−	−	−	+	−	−	−
N. meningitidis	+	+	−	−	−	+	−	−	−
N. sicca	+	+	+	+	−	−	d	+	d
N. subflava	+	+	d	d	−	−	+	d	d
N. flavescens	−	−	−	−	−	−	+	+	+
N. mucosa	+	+	+	+	−		d	+	+
N. lactamica	+	+	−	−	+		+	+	d

d = some strains positive, some negative.

Fig. 20-1. Electron micrograph of *Neisseria* (×27,000). (From A. S. Dajani, *Infect. Immun.* 14:776, 1976.)



2. All species parasitize the mucous membranes of humans.
3. All species are aerobic or facultatively anaerobic. The pathogens grow better in 5% to 10% CO_2.
4. All species produce the enzyme cytochrome oxidase. Members of the genus are said to be oxidase positive as determined by the oxidase test.

DIFFERENTIATION AMONG SPECIES OF *NEISSERIA*

The major species of the genus *Neisseria* and some of their differentiating properties are listed in Table 20-1. Establishing that an isolated bacterium is of the genus *Neisseria* is comparatively easy. It frequently becomes necessary, however, to distinguish between the closely related species within the genus. Nonpathogenic *Neisseria* microorganisms are almost always present in specimens obtained from the oral cavity and from the nasopharynx. There is an appreciable carrier rate of the meningococcus in the nasopharynx, and the meningococcus not infrequently is found in the genital tract and rectum of male homosexuals. The gonococcus does cause infections of the oral cavity and of the pharynx, infections that are usually relatable to oral-genital contact.

PROPERTIES OF THE *NEISSERIA* PATHOGENS: *N. MENINGITIDIS* AND *N. GONORRHOEAE*

FRAGILITY

The *Neisseria* pathogens, when apart from the host, die in environmental conditions in which most other bacteria are able to survive. They can survive only in a comparatively narrow temperature range; they autolyze readily. Because of their delicate nature, it is important that clinical specimens suspected of containing the pathogenic microorganisms be placed in suitable cultural conditions as soon as possible or be maintained in suitable transport media until they can be properly cultured.

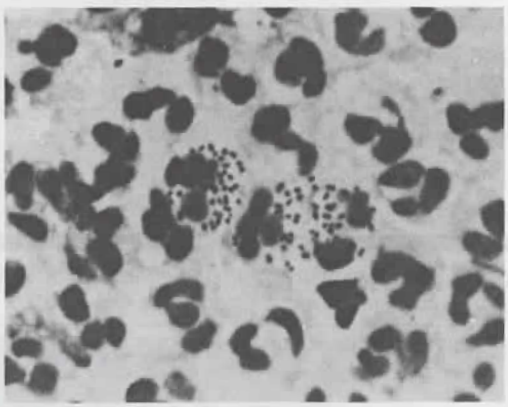
CULTURAL FASTIDIOUSNESS

Chocolate blood agar prepared with various supplements is a basic culture medium for the *Neisseria* pathogens. Thayer-Martin medium exemplifies the enriched media used for isolation and culturing. It can be made selective by the addition of a combination of chemotherapeutic agents symbolized by the letters *VCN*, for vancomycin, colistimethate, and nystatin. Vancomycin inhibits gram-positive cocci, colistimethate inhibits gram-negative bacilli, and nystatin inhibits yeasts. Modified Thayer-Martin medium also contains trimethoprim to better inhibit gram-negative bacilli.

REQUIREMENT FOR ELEVATED CO_2 LEVELS

The gonococcus requires an elevated CO_2 environment for successful laboratory growth. The meningococcus does not strictly have to have it, but it is recommended for isolation and for augmentation of its growth. CO_2 incubators are available. A practical but less reliable method to achieve an elevated CO_2 atmosphere is to place the inoculated plates and a candle in a wide-mouth jar, light the candle, and then seal the jar—the "candle jar" or "candle extinction" technique. The GasPak system (see Fig. 7-6) also can be used with a disposable CO_2 generator envelope.

Fig. 20-2. Smear of urethral pus containing gonorrhea organisms. In acute infections the paired, bean-shaped *Neisseria* appear mostly within the cytoplasm of neutrophils (*center*). The large, dark structures are the lobed nuclei of the neutrophils. The circular grouping of the gonococci is due to their confinement within the indistinct cytoplasmic membrane of the neutrophils. (From R. W. Thatcher and T. H. Pettit. Reprinted from the *Journal of the American Medical Association*, March 1, 1971, Volume 215, Copyright © 1971, American Medical Association.)



INTRACELLULAR HABITAT IN ACUTE INFECTIONS

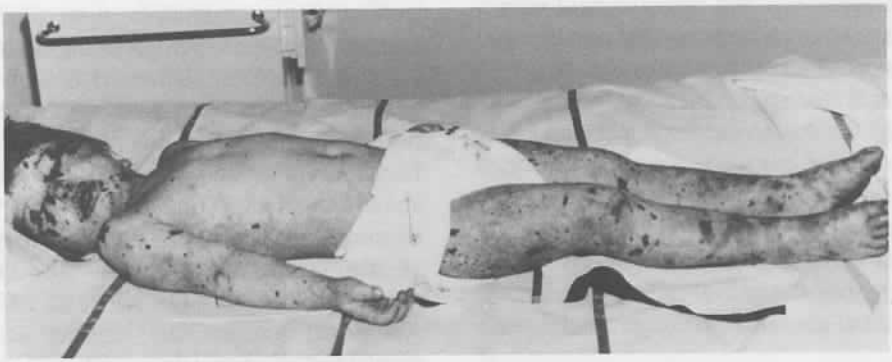
In smears of clinical material from an acute infection the gonococcus and the meningococcus characteristically appear in their distinctive morphology and grouping within the cytoplasm of cells, especially in neutrophils (Fig. 20-2).

NEISSERIA MENINGITIDIS— THE MENINGOCOCCUS

Meningococcal Disease

It is convenient to divide meningococcal disease into three clinical entities: nasopharyngitis, meningococcal septicemia, and meningococcal meningi-

Fig. 20-3. Purpura caused by severe meningococcal septicemia. (Courtesy B. Miller, Rietfontein Hospital, Edenvale, South Africa.)



tis. The latter two are the major invasive disease entities. Other forms of invasive meningococcal disease include septic arthritis, endocarditis, osteomyelitis, and pneumonia. There were about 2400 cases of meningococcal disease reported per year in the years 1978–1983 in the United States. Before the advent of serotherapy and chemotherapy, mortality on a worldwide basis was 50 to 80 percent in epidemics, 30 to 40 percent in sporadic cases, and neared 100 percent in infants. Presently mortality from the septicemic form is 20 to 30 percent, and from *meningitis* 2 to 10 percent.

NASOPHARYNGITIS. Infection of the nasopharynx is usually short-lived, frequently symptomless, and in fact is not regarded as a genuine clinical entity by some. Meningococci that are colonizing the nasopharynx can spread from this site into the bloodstream to cause meningococcal septicemia, or they can spread to adjacent sites and into the bloodstream to cause meningococcal meningitis.

MENINGOCOCCAL SEPTICEMIA. The term *meningococcemia* is used by many to signify meningococcal septicemia. *Meningococcemia* is probably more appropriately used to indicate the presence of meningococci in the blood, as occurs not only in septicemia but also in many patients who have meningococcal meningitis.

Infection of the blood can be accompanied by high fever, arthritis, blockages of small vessels, and a rash that starts as pinpoint hemorrhages (petechiae) of the skin and mucous membranes. In severe infections the skin rash becomes more extensive and takes the form of irregularly shaped, dusky red blotches (purpura—Fig. 20-3). A serious development is a fulminating sepsis that produces hemorrhagic necrosis in the cortex of both adrenal glands (adrenal apoplexy). The profound physiological effects of adrenal insufficiency and vascular effects lead to rapid collapse and death. Probably no other acute bacterial infection is as swiftly fatal as meningococcal septicemia. The complex of changes accompanying the bilateral adrenal cortical hemorrhage is termed the *Waterhouse-Friderichsen syndrome*, a syndrome that is most frequently associated with the meningococcus. The case fatality ratio in 1978 was 25.1 percent for 289 cases of meningococcemia.

MENINGOCOCCAL MENINGITIS. Meningitis can be caused by a variety of microorganisms and by noninfectious conditions such as cancer, systemic lupus erythematosus, and injected chemicals. Eighty-four percent of reported cases of bacterial (septic) meningitis in patients over one month old are caused by *Neisseria meningitidis, Streptococcus pneumoniae*, and *Hemophilus influenzae*, all three of which normally colonize the nasopharynx.

Because the meningococcus is the only microorganism to cause septic meningitis in epidemic form, it is designated the causal agent of epidemic meningitis. *Spinal meningitis* and *cerebrospinal meningitis* are terms used to indicate central nervous system infections caused by the meningococcus.

Meningococcal meningitis usually begins suddenly with high fever, severe headache, and pain and stiffness of the neck, back, and shoulders. Nausea and vomiting occur frequently. Petechial or purpuric lesions occur in about 50 percent of meningococcal meningitis patients. Survivors frequently suffer major residual effects (sequelae) such as deafness, mental retardation, and behavioral defects.

Epidemiology

The asymptomatic nasopharyngeal carrier is an important link in the transmission of meningococci. The carriage rate in the civilian population in interepidemic periods ranges from 2 to 8 percent. The rate increases markedly in community epidemic situations, in close contacts of patients, and in closed populations such as formerly occurred in military installations that processed recruits. There is a high carrier rate among homosexuals.

Occurrence of disease is highest in those 6–24 months old and in those 10–20 years old. Predisposing factors to meningococcal disease are poor general health, substandard or crowded living conditions, possibly IgM deficiency, and acute respiratory disease. Seasonal peaks occur in late winter and early spring.

Close contacts of persons with meningococcal disease are at increased risk (about 1000 times that in the general population) of developing meningococcal disease. Persons at highest risk are household contacts; day-care center contacts; medical personnel who have intubated, suctioned, or resuscitated diseased patients; and persons who have had intimate oral contact or who have shared food and beverages with infected persons. Nearly one-third of the contacts who develop meningococcal disease do so within 4 days after the index (source) patient is hospitalized.

Antigenic Composition

There are three ways in which the antigens of serologically and chemically different strains of meningococci are organized: serogroups, serotypes, and immunotypes. Serogrouping is based on chemical and structural characteristics of the meningococcal capsular polysaccharides. Serotyping is based on outer membrane proteins of the cell wall. Immunotyping is based on lipopolysaccharides and some chemically undefined antigens. Presently meningococci are referred to primarily on the basis of their serogroup antigens. The classical serogroups are A, B, C, D, X, Y, Z, 29E, and W135. Additional antigenically distinct serogroups lettered H, I, K, and L presently are only occasionally mentioned in the literature.

Laboratory Identification

ISOLATION, CARBOHYDRATE UTILIZATION TESTS, OXIDASE TEST. The organism may be isolated from blood, the nasopharynx, skin lesions, cerebrospinal fluid (CSF), and other sites such as the urethra and endocervix and the anal canal of homosexuals. Specimens are cultured on media such as supplemented chocolate agar and Thayer-Martin medium in an elevated CO_2 environment. The colony characteristics are observed. Gram-stained smears and carbohydrate utilization tests are conducted from colony sources. The cytochrome oxidase test (simply referred to as the oxidase test) is performed.

Cytochrome Oxidase Test. The cytochrome oxidase test can be performed several ways. One method is to place drops of the oxidase reagent (a solution of tetramethyl-*p*-phenylenediamine-dihydrochloride or dimethyl-*p*-phenylenediamine hydrochloride) directly onto suspected colonies and observe them for a color change that in a positive test progresses from pink to maroon or black. Another method is to remove part of a colony with a non-iron-containing implement (a flat toothpick works well) and apply the cells to a filter paper strip that has been impregnated with the oxidase reagent. The appearance of a purplish maroon color within seconds at the point of application is a positive reaction.

LABORATORY DIAGNOSIS OF MENINGOCOCCAL MENINGITIS. The cornerstone of diagnosis of all acute bacterial (septic) meningitis, not just of meningococcal meningitis, begins with a lumbar puncture to obtain CSF. The CSF in acute bacterial (septic) meningitis, as opposed to acute aseptic meningitis, is under increased pressure and cloudy, the white blood cell count is increased, protein is elevated, and glucose is reduced. Some of the CSF is Gram-stained for a presumptive diagnosis, and some is processed as described above under Isolation.

Serological Tests Performed on Cerebrospinal Fluid. The serological tests that may be performed directly on CSF include the capsular swelling (quellung) (see Fig. 19-10) reaction, the fluorescent antibody staining procedure, and counterimmunoelectrophoresis (CIE). Negative capsular swelling tests and negative CIE test results do not necessarily rule out a meningococcal infection because serogroups other than A and C frequently possess insufficient capsular antigens to be detected by these methods. Monoclonal antibodies against serogroup B are available, and they can be used to detect the bacterium in CSF, blood, and urine in 15 minutes. Serological tests such as agglutination can also be performed on fresh cultures.

Prevention and Control

CHEMOTHERAPY. The seriousness of meningococcal disease makes it essential to initiate treatment promptly; the use of antimicrobial drugs should not await the completion of laboratory studies. At one time the sulfa drugs were the drugs of choice but because there are now so many sulfonamide-resistant strains these drugs should not be used unless susceptibility is established by susceptibility tests. Penicillin is now the drug of choice; chloramphenicol is an alternative choice when there is sulfa drug resistance or penicillin hypersensitivity. Trials are currently in progress to assess the role of third-generation cephalosporins against the more common bacterial agents that cause meningitis. A combination of ampicillin and moxalactam is highly active against the major meningeal bacterial agents including the meningococcus.

ACTIVE IMMUNIZATION. Immunizing materials have been developed against serogroups A, C, Y, and W-135 meningococci. The antigens consist of highly purified, chemically defined polysaccharides. The vaccines are highly immunogenic and effective except that the group C vaccine does not induce protective levels of antibody in children younger than 2 years. All army and navy recruits beginning in October 1971 have received the group C polysaccharide vaccine. The immunization has virtually eliminated military-associated serogroup C disease.

Group B meningococci are poorly immunogenic, and consequently no vaccine is available for group B.

CHEMOPROPHYLAXIS. Chemoprophylaxis is recommended for close contacts (see under Epidemiology) of persons with meningococcal disease. Early use of rifampin is recommended unless the isolated meningococcus proves to be susceptible to sulfadiazine. An adjunct to chemoprophylaxis is active immunization. Over 50 percent of the high-risk close contacts who get a meningococcal infection develop the infection more than 5 days after contact with the primary case—sufficient time for the active immunization potentially to yield some benefit.

NEISSERIA GONORRHOEAE—THE GONOCOCCUS

There are now at least 16 sexually transmitted diseases (STD). The major ones were described in Chapter 13. It is not uncommon to find persons suffering from two or more of them at the same time. The most common sexually transmitted pathogens in the United States, *N. gonorrhoeae, Chlamydia trachomatis*, and herpes simplex virus (HSV) type 2 are believed to cause an estimated 10 million cases of STD annually.

The genital form of gonococcal disease is referred to in street language by such terms as "clap," "strain," and "gleet." The gonococcus generally is thought of as causing infections only of

Fig. 20-4. Purulent discharge typical of gonorrhea in men. Often it is only a drop of pus noticed on arising, but in severe cases the discharge is continuous and heavy. (From B. N. Morton, *VD: A Guide for Nurses and Counselors*. Boston: Little, Brown, 1976.)

the genitourinary (GU) tract and associated structures, but extragenital gonococcal infections manifested in such forms as arthritis, pharyngitis, dermatitis, proctitis, and septicemia are not all that uncommon.

Gonococcal Diseases

GENITOURINARY TRACT INFECTION IN THE MALE. Infection in men usually takes the form of acute urethritis. Among the early symptoms are sensations of discomfort and pain. The inflammatory response initially triggers a mucoid discharge followed by a purulent exudate, usually in 2–5 days (Fig. 20-4). The infection can progress from the anterior urethra to the posterior urethra in 10–14 days, with increasing dysuria (difficult urination), polyuria (frequent urination), and occasionally general symptoms including headache and fever. The glands, ducts, and vesicles of the male GU tract may become sites of local complications. Chronic infections of the prostate, seminal vesicles, and epididymides may ensue. Urethral strictures may occur.

At one time it was believed that asymptomatic infections did not occur in men, but asymptomatic infections are now known to occur in an estimated 5 to 20 percent of the infected male population.

Fig. 20-5. The discharge caused by gonorrhea in women is noticeable on the cervix rather than on the vaginal walls. The test sample is taken from the cervical os, the small opening at the center of the cervix. (From B. N. Morton, *VD: A Guide for Nurses and Counselors*. Boston: Little, Brown, 1976.)

GENITOURINARY TRACT INFECTION IN THE FEMALE. Acute gonorrhea may involve the urethra, Skene's and Bartholin's glands, and the endocervix (Fig. 20-5). The endocervical glands are the traditional site of infection, but the rectum is also a common site. Exudate from the endocervix probably contaminates the perineum, and the microorganisms spread to the rectal mucosa. There is no gonococcal vaginitis in the postpubertal female. Symptoms of acute infection of the lower tract are seldom severe. The most prevalent symptoms of acute infection are abdominal or pelvic pain, vaginal discharge (which originates in the endocervix), and dysuria. Chronic infections may be indicated by tenderness of the lower abdomen, backaches, low-grade inflammations of the urethra and GU tract-associated structures, and profuse menstrual flow. Asymptomatic carriers present a major obstacle in controlling the spread of gonorrhea.

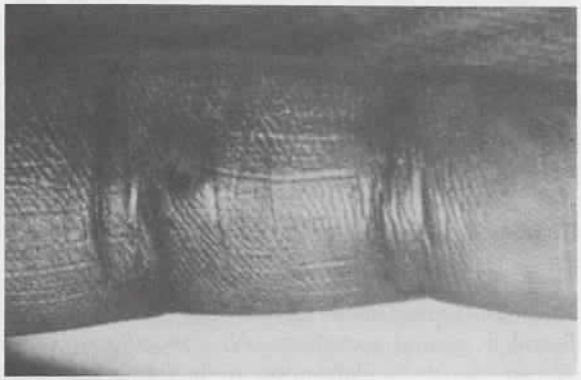
Pelvic Inflammatory Disease, Upper Tract Disease. There may be an ascending spread of gonococci from the endocervical glands along the endometrium (the inner mucous membrane of the

uterus) to the fallopian tubes (where it causes salpingitis) and from there to adjacent structures. The ascending infection is termed *upper tract disease* or *pelvic inflammatory disease* (PID). PID is caused by other agents, such as *C. trachomatis*, anaerobic bacteria (*Bacteroides* and gram-positive cocci), *Actinomyces israelii*, and *Mycoplasma hominis*. It is estimated that 30 to 80 percent of cases of PID are gonococcal, but polymicrobial PID is quite usual. Upper tract disease most often occurs within 7 days of the previous menstrual period. Signs and symptoms typical of acute upper tract disease include chills, fever to 102°, severe bilateral lower abdominal pain, and rebound tenderness (pain on the release of pressure). Those who experience recurrences are at risk of infertility and ectopic pregnancies.

EXTRAGENITAL INFECTIONS. *Local Infections*. Local infections occur in various areas of the body, determined usually by the mode of contact: Pharyngitis, conjunctivitis, and proctitis are examples. Blindness in the newborn can develop from infection of the eyes as the child passes through the birth canal of an infected mother.

Disseminated Gonococcal Infection. Gonococci from primary sites of infection (endocervix, rectum, pharynx, male urethra) can spread via the bloodstream (hematogenous route) to cause most commonly an arthritis-dermatitis syndrome and occasionally meningitis or endocarditis. One to three percent of individuals with genital infections develop disseminated gonococcal infection (DGI). Those who develop DGI are usually asymptomatic in regard to the primary site of infection. Fever, chills, myalgia, and malaise are nonspecific symptoms of the bacteremic phase of DGI. Between 50 and 70 percent of patients with DGI have dermatitis and more than 90 percent have arthropathy. Active skin lesions are usually found in those who have joint involvement (Fig. 20-6). An average of 5 to 30 lesions appear, mostly on the extremities, and they progress from red papular lesions to pustular, hemorrhagic, or necrotic lesions. The presence of such lesions in various stages of development in sexually active individuals should lead the physician to suspect and to rule out DGI. The arthritis is usually felt in several joints, with knees, ankles, and wrists being the most frequently involved. Joint destruction can occur if the infection is left untreated.

Fig. 20-6. Example of a skin lesion caused by gonococcemia. Metastatic gonorrhea may cause arthritic symptoms accompanied by red papules, vesicles, or pustules typical of those shown. (Courtesy N. J. Fiumara, Massachusetts Department of Public Health, Division of Communicable and Venereal Diseases.)



Epidemiology

Except for vulvovaginitis in prepubertal (usually institutionalized) girls and conjunctivitis in the newborn, gonococcal infections are usually spread by sexual contact. The annual incidence of gonorrheal infections in the United States is estimated at 2.5 million. In a screening program conducted in 1978, 4.7 percent of the 8,641,188 specimens obtained from women at a variety of health care facilities were culturally positive for the gonococcus. This figure exemplifies the extent of infection in the population and the enormity of the control problem.

Among the factors that predispose to the epidemic proportions of gonorrhea are high degree of transmissibility; short incubation period; high rate of asymptomatic carriers, especially women; lack of lasting immunity; increasing resistance to antibiotics; and, conceivably, changes in sex mores and practices.

Virulence Factors

The pathogenic mechanisms of the gonococcus are not well understood. The following factors are currently regarded as possible agents of gonococcal pathogenicity:

PILI. Gonococci produce four colony forms: T1, T2, T3, and T4. Only the bacteria in T1 and T2 colonies are piliated, and they are regarded as virulent because they produce infection in volunteers. Pili apparently represent only one type of adhesin involved in pathogenicity (see Chapter 13 for further discussion of epithelial adherence and penetration by the gonococcus).

IgA PROTEASE. Strain-specific secretory IgA is found in genital secretions. *N. gonorrhoeae* (also *N. meningitidis*) elaborates an IgA protease that cleaves and inactivates the secretory IgA subclass IgA1. Nonpathogenic *Neisseria* species evidently do not produce IgA protease.

CAPSULE. Three different groups of investigators in 1977 reported the presence of a capsule on freshly isolated strains of the gonococcus. The role of the gonococcal capsule as a virulence factor is uncertain at this time.

EPITHELIAL ENDOCYTOSIS. The persistence of the gonococcus in the carrier state may be due to epithelial endocytosis. Gonococci in carriers have been found in epithelial cells obtained from the cervix. Apparently the gonococci attach to the epithelial cells of the cervix, are subjected to endocytosis, multiply within the cell, and are protected from the phagocytic activities of leukocytes.

ENDOTOXINS. Gonococcal endotoxin is not as apparent a virulence factor as are the endotoxins of some of the other gram-negative bacteria when the gonococcal infection is a mucosal one. Gonococcal endotoxin, however, is probably responsible for some of the pathophysiological effects observed in DGI.

Laboratory Diagnosis

Laboratory identification procedures for *N. gonorrhoeae* include the following:

EXAMINATION OF STAINED SMEARS OF CLINICAL MATERIAL. The value of Gram-stained clinical specimens varies with the site from which the specimen was obtained and with the sex of the patient. The finding of intracellular gram-negative diplococci in smears of urethral pus is viewed as presumptive evidence of gonorrheal infection in men. Smears of specimens from the female genital tract are much less reliable for diagnostic purposes; acute infections are not nearly as apparent as in the male, and other gram-negative diplococci and other organisms that resemble the gonococcus may appear in such specimens. Smears from the pharynx or rectum have little reliable diagnostic value.

IDENTIFICATION PROCEDURES CONDUCTED ON ISOLATES. In order to diagnose a gonorrheal infection reliably, it is necessary to culture out the organism to establish its identity. It is important to give careful attention to the collection of appropriate specimens and to the appropriate processing of the specimen. Oropharyngeal and anorectal specimens should also be obtained along with the regularly collected urethral and endocervical specimens when there is reason to believe that oral or anal contact has occurred. Colonial and microscopic characteristics, the oxidase test, and carbohydrate (CHO) metabolism tests are the principal observations and tests that are routinely conducted on the isolated organisms.

Colony Types. N. gonorrhoeae displays four colony types. Colony types T1 and T2 are small, sticky, and dark (when obliquely lighted); they develop in primary culture from clinical material. T1 and T2 colonies are correlated with virulence.

Carbohydrate Metabolism Reactions. The characteristic pattern of CHO metabolism has been the major confirmatory test in many laboratories. The CHO tests have shortcomings because oxidative organisms such as *Neisseria* produce less acid than do fermentative organisms. Positive reactions develop slowly, and the concomitant production of ammonia from peptones in the medium may neu-

tralize the acids that are produced. Reliable, rapid, miniaturized multitest systems for the identification of *Neisseria* are now commercially available. The CHO aspect of the multitest systems can produce results in 4 hours because the tests are designed to produce reactions that are based on preformed enzyme activity rather than waiting for sufficient growth to first occur.

Superoxol (Catalase) Test. The Superoxol test, which is like the conventional catalase test except that the concentration of hydrogen peroxide (H_2O_2) is 30% rather than 3%, is an inexpensive, rapid screening test for organisms growing on Thayer-Martin medium. Any organism that grows on this medium and that is Superoxol positive (bubbles of oxygen appear when the reagent is applied to colonies) is likely to be *N. gonorrhoeae*.

SEROLOGICAL TESTS. There are two generally accepted serological tests used in the identification of *N. gonorrhoeae*: the direct fluorescent antibody technique and the staphylococcal coagglutination technique. The latter technique employs a strain of protein A–containing staphylococci that are coated with antigonococcal antibodies (see under Protein A in Chapter 19). Many other serological test techniques have been researched, but in many cases the serological test positivity rate does not correspond well enough with cultural positivity or the false-positive rate is too high. A dependable, easily performed serological test would be especially useful in detecting carriers. The public health control problem does not lie with the apparent cases of gonococcal infection but with the detection of the asymptomatic carriers.

LIMULUS LYSATE TEST AS AN EXCLUSION TEST FOR CERVICAL GONORRHEA. The limulus lysate test is a test that is exquisitely sensitive for detecting bacterial endotoxins. Limulus lysate is an extract prepared from the circulating blood cells (the blood cells are called amoebocytes) of the horseshoe crab (*Limulus polyphemus*). The lysate gels in the presence of minute amounts of endotoxin. Investigations indicate that the limulus lysate test can be used as an exclusion test for females who have had sexual contact with a male known to be infected with the gonococcus. A negative test with samples obtained from the cervix correlates with the absence of cervical gonorrhea.

Chemotherapy

NEW DEVELOPMENTS. Several recent observations and reports affect the treatment recommendations. There is a high frequency—as high as 45 percent in some studies—of cotransmitted and coexisting gonococcal and chlamydial infections. Concern is growing about the serious complications that accompany these infections. The incidence of infections caused by penicillinase-producing *N. gonorrhoeae* (PPGN) is increasing, and there are reports of emerging tetracycline-resistant and spectinomycin-resistant strains. On the plus side, new antimicrobials, particularly new cephalosporins, are becoming available that appear to be effective in the treatment of gonococcal disease. (See boxed essay, p. 482.)

CHEMOTHERAPY FOR UNCOMPLICATED GONOCOCCAL INFECTION. Several forms of penicillin and tetracycline are the most widely used antibiotics in uncomplicated infections, that is, urethral, cervical, pharyngeal, and anorectal infections.

The forms of penicillin that are used are aqueous procaine penicillin G (APPG) and amoxicillin or ampicillin. The penicillins have two major advantages: Only a single dose is required, so there is no problem with patient compliance, and penicillin aborts incubating syphilis. The major disadvantages are the ineffectiveness against chlamydial infections and the penicillin hypersensitivities of some patients. Tetracyclines are effective against coexisting chlamydial infections, but there is a patient compliance problem because the patient must take 2 to 4 doses per day for 7 days. A drug regimen consisting of a single dose of amoxicillin or ampicillin combined with tetracycline that is taken on the regular multiple-dose schedule is being evaluated.

Prophylaxis of Ophthalmia Neonatorum

Most states have a law that requires the instillation of a prophylactic agent into the eyes of newborn in-

GLOBAL DISTRIBUTION OF PENICILLINASE-PRODUCING *NEISSERIA GONORRHOEAE*

Strains of penicillinase- (beta-lactamase–) producing *Neisseria gonorrhoeae* (PPNG) continue to spread throughout the world. The number of countries where PPNG has been identified through May 1981 appears to be limited only by the capacity of local laboratory services to isolate and test for these strains. Many countries with good surveillance systems have observed a two- to sixfold increase in the number of such cases reported within the last 18–24 months.

Gonococcal strains partially resistant to penicillin and other antibiotics have long been recognized, and infections caused by them have been treated with dose increases or with alternative antibiotics. However, many areas of the world now have a high proportion of patients for whom penicillin therapy is not effective because of gonococcal strains with plasmid-mediated resistance. Alternative regimens for effective treatment may be difficult to identify and may result in increased treatment costs to the point where many governments or patients can no longer afford such treatment. Because alternative therapies are expensive, less effective treatments such as penicillins continue to be used. This further selects for drug resistance and extends the infectious period for patients. Thus gonorrhoea transmission may be expected to continue, and the proportion of infected patients who develop complications may be expected to rise.

Continued efforts to control PPNG transmission in this country must include testing of all gonococcal isolates for penicillinase production; prompt identification of sexual partners of all PPNG patients; screening of all groups considered to be at high risk of PPNG infection; and treatment of all the following with 2 g of spectinomycin: all PPNG patients and their sexual partners; patients who acquired gonorrhea in countries with high PPNG prevalence; and all patients for whom penicillin, ampicillin, amoxicillin, or tetracyclines are not effective treatment for gonorrhea.

From *MMWR* 31:1, 1982.

fants immediately postpartum. Silver nitrate solution 1% (Credé's method) is effective for gonococcal ophthalmia neonatorum but is not effective against chlamydial conjunctivitis. Either erythromycin (0.5%) ophthalmic ointment or tetracycline (1.0%) ointment is effective against both agents.

Immunity

IMMUNE SYSTEM RESPONSE. Long-lasting immunity does not appear to develop as a result of natural infections with *N. gonorrhoeae*. Repeated infections occur in an individual, and an acute infection may be superimposed on a chronic one. Antigonococcal

secretory IgA is found, however, in genital secretions. Serum antibody formation takes place. Bactericidal serum antibodies can be demonstrated, and several serological tests developed in recent years detect antibodies to *N. gonorrhoeae*. The injection of a suspension of killed gonococci into the skin can elicit a positive skin reaction. Why the failure, then to develop a solid immunity?

FAILURE TO DEVELOP A SOLID IMMUNITY. Failure to develop a solid immunity could well be due to the heterogeneity of the gonococcal antigens. Strain-specific secretory antibodies evidently are produced, but they are ineffective against the many other strains. Antigenic variability is found in the pili and in the recently discovered outer membrane proteins; it probably exists in the newly described capsule. Sixteen outer membrane protein serotypes have already been distinguished. They are similar to the strain-specific M proteins of the streptococci. Another possible reason for failure of solid immunity to develop is that the first encounter with the gonococcus is at the mucosal surface, where the immunity owing to secretory IgA tends to be less solid than serum immunity. That situation, coupled with antigen diversity and the elaboration of IgA-destructive protease by the gonococci, may account for the lack of protection against reinfection.

PROSPECTS FOR A VACCINE. According to vaccination studies in experimental animals, it is possible to increase resistance to the same gonococcal strain approximately 1000-fold. Humans evidently produce a rather high level of strain-specific secretory IgA, which prevents the attachment of the gonococcus. In view of the protection against only the specific strain, a successful vaccine will probably have to be polyvalent—that is, contain immunogenic antigens from a number of strains. The antigens that are prominent in stimulating immunity will have to be identified and incorporated into the polyvalent vaccine. The likely sources of those antigens are the pili, the capsule, and the outer membrane proteins.

OTHER MEMBERS OF THE FAMILY NEISSERIACEAE

NONPATHOGENIC *NEISSERIA* SPECIES

Members of the genus *Neisseria* (see Table 20-1) other than *N. gonorrhoeae* and *N. meningitidis* are not frequently involved in overt disease. Their importance lies in the following facts: (1) They are the second most prevalent aerobic microorganisms in the oral cavity and upper respiratory tract, and some are occasionally recovered from genital sites; (2) there are times when it is necessary to differentiate them from the pathogens; (3) occasionally they are found as causal agents of infections such as meningitis, endocarditis, pneumonia, osteomyelitis, and septicemia.

ACINETOBACTER

Acinetobacter is a genus with many species of gram-negative diplococci or coccobacilli. Its members are widely distributed in nature and are often part of the normal human flora, especially that of the skin. There is one recognized species, *Acinetobacter calcoaceticus* (which includes the former *Herellea vaginicola* and *Mima polymorpha*), that is being reported with increasing frequency as an opportunist that causes a variety of infections, especially hospital-associated infections: pneumonia, bacteremia, nongonococcal urethritis, meningitis, and wound infections.

BRANHAMELLA

Branhamella catarrhalis is a gram-negative diplococcus formerly known as *Neisseria catarrhalis*. It is commonly found in the human nasopharynx. Occasionally it causes mucous membrane inflammation or pneumonia.

MORAXELLA

Members of *Moraxella* (six recognized species) are plump, gram-negative bacilli. *Moraxella lacunata* occasionally causes conjunctivitis in humans.

SUMMARY

The *Neisseria* are gram-negative diplococci that colonize, and sometimes infect and cause disease in, the mucous membranes of humans. Members of the genus are cytochrome oxidase positive and that, coupled with their gram-negative diplococcal features, differentiates *Neisseria* from other genera. Differentiation of species within the genus primarily is based on carbohydrate utilization. The two pathogens, *N. meningitidis* (meningococcus) and *N. gonorrhoeae* (gonococcus), are delicate, culturally fastidious microorganisms. Special culture media such as supplemented chocolate agar or Thayer-Martin medium and CO_2 elevation (candle jar) are used for successful cultivation. The pathogens typically appear in the cytoplasm of neutrophils in acute infections.

The two best known meningococcal infections are septic meningitis, which can occur in epidemic form, and a fulminant meningococcemia that is rapidly fatal. There are nine serogroups: A, B, C, D, X, Y, Z, 29E, and W135. Vaccines against A and C are available. Penicillin, with choramphenicol as an alternative, is used when there is sulfa drug insusceptibility.

Gonorrhea is a major sexually transmitted disease (STD). Acute genital infections in men are usually manifested as an urethral exudate. The primary site of genital infection in women is the endocervical glands. The development of pelvic inflammatory disease (PID) is not uncommon. Asymptomatic infections are a problem in controlling infections. The site of localized extragenital infections (pharyngitis, proctitis, conjunctivitis) usually is determined by mode of contact. Disseminated gonococcal infections (DGI) are spread via the hematogenous route and mostly take the form of an arthritis-dermatitis syndrome. Imputed gonococcal virulence factors are pili, IgA protease, capsules, intraendothelial cell habitat, and endotoxins. Chemotherapy is somewhat varied and is for one thing based on whether the infection is uncomplicated or complicated (e.g., by pregnancy or youth). Several forms of penicillin and tetracycline are the principal chemotherapeutic agents. $AgNO_3$ 1% is used prophylactically in neonates to prevent blindness.

QUESTIONS FOR STUDY

1. How is the genus *Neisseria* differentiated from other genera of bacteria?
2. What is the principal habitat of *Neisseria* in humans?
3. What is the principal laboratory mode of differentiating species within the genus *Neisseria*?
4. What is the candle jar technique, and why is it used?
5. Which three bacterial species collectively account for a high percentage of cases of septic meningitis?
6. The microscopic examination of which clinical material is useful for identifying an acute infection by the *Neisseria* pathogens? What is the typical finding?
7. What is meningococcemia?
8. Name the serogroups of *N. meningitidis*. Against which of these is there a vaccine?
9. What is the principal site of gonococcal genital infections in women?

10. Describe pelvic inflammatory disease (PID), and name the two principal causal agents.
11. When DGI occurs what is the usual source in the body of the gonococci, and how do they spread? What is the principal clinical form of DGI?
12. What are the principal drugs used in chemotherapy for uncomplicated gonococcal infections?

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21. THE GRAM-POSITIVE SPOREFORMERS: THE BACILLI AND CLOSTRIDIA

OBJECTIVES

To describe the types of disease caused by the genus *Bacillus* and the virulence factors associated with them

To discuss the techniques used to treat and control disease caused by *Bacillus anthracis*

To list the major species of *Clostridium* and the diseases they cause in humans

To explain the various laboratory techniques used to differentiate species of *Clostridium*

To explain the mechanism of action of the clostridial toxins

To describe the methods used to treat and prevent the various clostridial infections

Bacillus
General Characteristics
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Cultivation
Antigenic Composition
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Diseases Caused by the Pathogenic Clostridia
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Clostridium botulinum Toxin
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Gas Gangrene
Clostridium perfringens Toxins
Pathogenesis
Laboratory Diagnosis
Treatment and Prevention
Food Poisoning (*Clostridium perfringens*)
Tetanus
Tetanus Toxins
Pathogenesis
Laboratory Diagnosis
Treatment
Prevention
Other Clostridial Conditions

The gram-positive sporeformers comprise two major genera of bacteria: *Bacillus* and *Clostridium*. In culture they appear as long rods varying in length between 3 and 8 μm. In the spore state they are relatively resistant to heat and many chemicals. Autoclaving for 15 minutes at 120°C and 15 pounds per square inch pressure is the most effective way to destroy them. Most species exist as saprophytes in soil, water, and vegetation although some are harbored in the intestinal tract of animals and humans. Only a few species are pathogenic to humans. They are, however, among the most virulent.

BACILLUS

Practically all species of *Bacillus* are found as saprophytes in soil, water, and vegetation. Only *B. anthracis* is a major pathogen. (*B. cereus* has been associated with food poisoning, but only a few cases have been recorded.) *B. anthracis* is the causative agent of anthrax, a disease that occurs primarily in cattle but can be transmitted to humans.

GENERAL CHARACTERISTICS

Morphology and Staining

The anthrax bacillus is a nonmotile facultative anaerobe 4 to 8 μm in length and 1.0 to 1.5 μm in width. The spore, located in the center of the cell (endospore), is produced only when the cells are cultivated outside the animal host tissue. The colonies that develop on an agar plate are large, opaque, and white. The edge of the colony gives the appearance of undulating bands and is referred to as "medusa head."

The vegetative cells are easily stained with ordinary laboratory dyes. Smears prepared from culture appear as long chains of rods and with their centrally located refractile (colorless) spores look like chains of pearls. Smears prepared from tissue

show very short chains of encapsulated bacteria. The entire chain is surrounded by what is apparently one large capsule. Only the virulent strains produce a capsule in vivo. Avirulent strains may produce a capsule in vitro but not in vivo.

Cultivation

B. anthracis is easily cultivated on ordinary laboratory media with maximum growth obtained under aerobic conditions. When cultivation takes place in an atmosphere of CO_2, a capsule is produced. The organism does not lyse red blood cells, and this property is used in identification procedures.

Antigenic Composition

Three antigens from the anthrax bacillus have been identified in the laboratory.

CAPSULAR POLYPEPTIDE. Most encapsulated microbes have capsules composed of polysaccharide. *B. anthracis* is only one of a few microorganisms that produce a polypeptide capsule (polyglutamic acid). The capsular material, which is antiphagocytic, is associated with virulence since nonencapsulated strains are avirulent. The capsular material does not stimulate the production of protective antibodies.

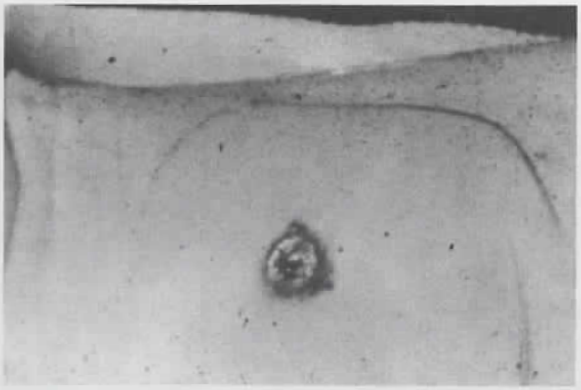
SOMATIC POLYSACCHARIDE. Somatic polysaccharide is found in the cell wall and is not a virulence factor. Protective antibodies are not produced against it.

PROTEIN EXOTOXIN COMPLEX. The protein exotoxin complex is excreted by the cell and is composed of three fractions; edema factor, protective antigen, and lethal factor. The protective antigen is the major component in the currently licensed human anthrax vaccine.

PATHOGENESIS

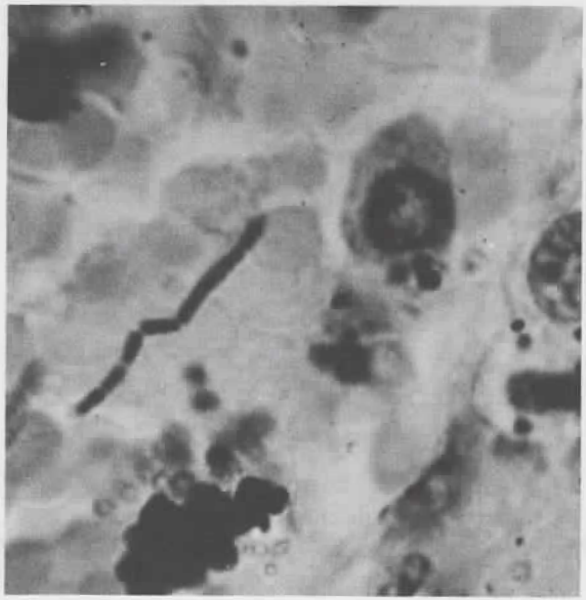
Anthrax is primarily a disease of herbivorous animals: sheep, horses, and cattle. The spores, which are found in the soil and on vegetation, are ingested or inhaled and gain entrance to the subcutaneous tissue through abraded skin or mucosa. After the spores germinate in the tissue, the vegetative cells produce an exotoxin that, in combination with the capsule, inhibits phagocytosis. If the bacilli reach the lymph and bloodstream, fatal septicemia may develop.

Fig. 21-1. Cutaneous anthrax. Lesion containing a central dark area (eschar) is observed on the forearm. (Courtesy P. Brachman.)



In humans the disease is considered an occupational hazard for those working in areas where livestock is handled or where animal hides and hair are processed and in microbiology laboratories. Inhalation of anthrax spores can result in a condition referred to as *woolsorters' disease*. The inhaled spores settle in the respiratory tract, producing local hemorrhaging and edema. Septicemia sometimes occurs, leading to meningitis. The most common form of the disease in humans, however, is called *cutaneous anthrax*. The organisms gain entrance to the body by contact with an abraded portion of the skin (Fig. 21-1). The fully developed lesion appears as a dark necrotic area surrounded by a rim of edema. In severe cases the regional lymph nodes may enlarge, and once the blood is invaded the disease may prove fatal. Gastrointestinal anthrax is uncommon in the United States but occurs more frequently in underdeveloped countries

Fig. 21-2. Appearance of anthrax bacillus as observed in tissue. (Courtesy Armed Forces Institute of Pathology, AFIP 28-59.)



where contaminated meats are sold. The toxin produced in the intestinal tract forms a necrotic lesion in the ileum or cecum. Case fatality rates for this condition are very high.

IMMUNITY

Recovery from infection by *B. anthracis* brings permanent immunity for humans and animals. Protective antibodies are produced as a result of the host's response to the exotoxin complex. At present the only vaccine safe enough for human use is a toxoid prepared from a fraction of the exotoxin. Animals can be immunized with live spore vaccine; the Sterne strain vaccine is prepared from avirulent anthrax spores.

LABORATORY DIAGNOSIS

The anthrax bacillus can be cultured from the soil, cutaneous lesions, the respiratory tract, or blood (Fig. 21-2). The appearance of other sporeforming bacilli from some of these sites occasionally makes identification difficult. The criteria for identification should include (1) Gram's stain characteristics, (2) appearance of the capsule, (3) appearance of nonhemolytic colonies on blood agar, (4) absence of motility, and (5) susceptibility to gamma phage and penicillin. Since the bacillus is so pathogenic for laboratory animals (the LD_{50} for mice is less than five spores), animal inoculation tests can also be used for positive identification.

TREATMENT

Penicillin is often the drug of choice, but streptomycin, tetracycline, and erythromycin are also effective against the bacillus. Treatment before the appearance of bacteremia is important because the antibiotics are not effective against the toxin.

PREVENTION

Anthrax is endemic in many areas of the world, especially the underdeveloped ones. Epizootic outbreaks still occur in the United States when unusual climatic conditions such as prolonged and heavy rains prevail. The most effective control program is vaccination of cattle, cremation or burial with quicklime of infected animals, and restricted movement of livestock.

Several cases of cutaneous anthrax are recorded each year in the United States. Many of them result from contact with goat hair or with animal hides that have not been decontaminated. Overseas travelers buying articles made of animal products must be made aware of these potential sources of infection. (See boxed essay, p. 493.)

Control of the disease in humans whose occupation may be a predisposing factor is provided by immunization with the toxoid.

OTHER BACILLI

Because of ease in cultivation, nonpathogenicity, and heat resistance, the spores of this group of bacteria are routinely used in the laboratory for experimental and testing procedures. *B. subtilis* is employed as a test organism to measure the effectiveness of ethylene oxide sterilization. Spores of *B. stearothermophilus*, because of their unusually high resistance to heat, are employed to test the efficiency of autoclaving. Finally, spores of *B. pumilus* are used to test the efficiency of sterilization by ionizing radiation.

B. cereus is a ubiquitous microorganism that is frequently found in food. It is recognized as an important cause of foodborne disease in Europe but not in the United States. The reason *B. cereus* is infrequently associated with disease in the United States is because it is seldom sought. Disease is similar to other foodborne diseases, and if the cause is not confirmed epidemiologists presume other microbial agents are involved. Food poisoning by *B. cereus* results in two syndromes, one manifested by diarrhea and the other by vomiting. Each is caused by different enterotoxins. The one causing diarrhea is believed to act like cholera toxin, that is, it activates adenyl cyclase (see Chapter 25). Outbreaks in the United States are frequently associated with rice and rice products.

CLOSTRIDIUM

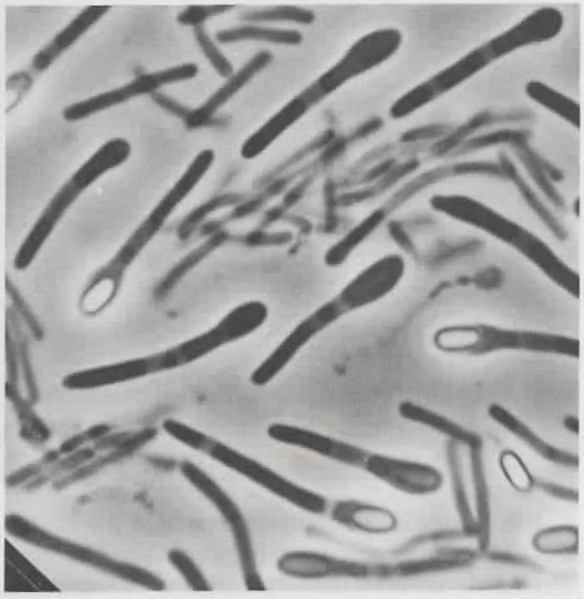
The genus *Clostridium* includes many species that inhabit water, soil, and vegetation and play a major role in animal and plant putrefaction. A few species parasitize the intestinal tract of humans and animals. Some of the species from these habitats are pathogenic and are the causative agents of botulism, tetanus, and gas gangrene.

GENERAL CHARACTERISTICS

Morphology

All clostridia are gram-positive sporeforming rods varying in length from 3 to 8 μm and in width from 0.4 to 1.2 μm. In most species the sporangia (vegetative cells with the enclosed spores) appear swollen (Fig. 21-3). With few exceptions the spores of this genus do not germinate unless suitable reducing conditions are available and oxygen is absent. The spores are unusually resistant to heat and can withstand temperatures of 120°C for as long as 10–15 minutes.

Fig. 21-3. Phase-contrast micrograph (×3600) of the endospores of a species of *Clostridium*. Note the refractile spore body and the swollen appearance of the vegetative cell. (Courtesy P. C. Fitz-James.)



Cultural and Biochemical Features

The biochemical and cultural properties of this genus are also used to distinguish the species within it. These properties include anaerobiosis, fermentation, and enzyme and toxin production.

ANAEROBIOSIS. The clostridia are considered obligate anaerobes. However, their response to oxygen varies considerably, depending on the concentration of oxygen, the oxidation-reduction potential of the medium, and other chemical and physical fac-

Table 21-1. Fermentative and Biochemical Properties of the Major Species of *Clostridium*

Species	Disease	Motility	Aerobic growth	Milk	Fermentation				Nitrate reduction	Indole formation
					Glucose	Maltose	Lactose	Sucrose		
C. botulinum	Botulism	Yes	No	A	+	+	−	V	−	−
C. tetani	Tetanus	Yes	No	C	−	−	−	−	−	+
C. perfringens	Gas gangrene	No	No	ACG	+	+	+	+	+	−
C. novyi	Gas gangrene	Yes	No	CG	+	+	−	−	−	−
C. histolyticum	Gas gangrene	Yes	V	CD	−	−	−	−	−	−
C. septicum	Gas gangrene	Yes	No	ACG	+	+	+	−	+	−

+ = positive reaction; − = negative reaction; A = acid; C = curd; G = gas; D = digested; V = variable.

tors. It is now believed that the superoxide radical produced during oxygen metabolism is the major factor responsible for oxygen toxicity (see Chapter 7).

The clostridia are easily cultivated in the laboratory when anaerobic conditions prevail. The most commonly used method for isolation of anaerobes is streaking blood agar plates and incubating anaerobically for 48–72 hours. After incubation the colonies should be examined for hemolysis, colony structure, and swarming of motile cells. Many specimens suspected of containing clostridia are often contaminated with gram-negative species, and primary isolation on plates incubated anaerobically is difficult. Some alternatives to primary isolation are as follows: (1) The specimen is heated to 80°C for 10–15 minutes to destroy any vegetative bacteria and then incubated at 37°C before being subcultured on laboratory media. (2) The specimen (except for some strains of *C. tetani*) is placed in tubes of thioglycolate broth containing glucose, incubated for 24–48 hours, and subcultured. In the thioglycolate broth the clostridial species ferment glucose, and the acids produced inhibit the growth of gram-negative organisms. (3) The specimen is transferred to chopped meat medium and sealed with petrolatum. The latter procedure is routinely used in isolation of anaerobic bacteria, especially when they appear to be in small numbers in the specimen. After suitable incubation at 37°C a subculture is made on other media.

FERMENTATION. The fermentation of various sugars is an important characteristic used in separating species of clostridia. Table 21-1 illustrates some fermentative and biochemical properties of the major species. In addition, some clostridia produce a considerable amount of gas during growth, a property that may be used in identifying *C. perfringens* (*C. welchii*). When milk is inoculated with this organism, the clotted milk is torn apart by the accumulated gas ("stormy fermentation").

ENZYME AND TOXIN PRODUCTION. An unusually large number of enzymes and toxins have been recovered from the filtrates of cultured clostridial species. They include hemolysins, collagenase, lipase, deoxyribonuclease, hyaluronidase, and the two potent exotoxins that cause tetanus and botulism. Many of these products are helpful in the identification or separation of species within the genus.

DISEASES CAUSED BY THE PATHOGENIC CLOSTRIDIA

Food Poisoning (Botulism)

Botulism is a noninfectious disease that results

ANTHRAX CONTAMINATION OF HAITIAN GOATSKIN PRODUCTS

Recent tests of Haitian handicrafts made from goatskin revealed that 6 of 22 (27 percent) samples were contaminated with *Bacillus anthracis*. The test items were collected at the Miami, New York, and San Juan quarantine stations in 1980 and 1981. Similar handicrafts were found to be frequently contaminated in 1974 (26 percent) and in 1976–1977 (55 percent).

Importation from Haiti of goatskins or products made in part or whole from goatskin with attached hair has not been permitted at U.S. ports of entry since April 19, 1974. Prohibited handicrafts include a variety of products such as drums, mosaic pictures, purses, rugs, and voodoo dolls. The ban was implemented after a Florida resident acquired anthrax from a contaminated goatskin drumhead. Commercial importation of raw goatskins for tanning in this country is not restricted.

From *MMWR* 30:338, 1981.

from the ingestion of preformed toxin in foods. About 25 percent of the incidences of clostridial food poisoning can be directly attributed to the species *C. botulinum*. *C. perfringens*, the primary causal agent of gas gangrene, is involved in the remainder of clostridial food poisonings. Recent evidence indicates that *C. botulinum* may also cause conditions other than food poisoning.

CLOSTRIDIUM BOTULINUM TOXIN. *C. botulinum* produces eight immunologically distinct neurotoxins (A, B, C_1, C_2, D, E, F, and G), permitting its separation into types. Types A, B, E, and F are responsible for human food poisonings. The toxins of some nonproteolytic strains are not completely active unless activated by proteolytic enzymes such as trypsin. Proteolytic strains produce their own enzymes to activate the toxin.

Type A toxin, which is responsible for most outbreaks of food poisoning associated with *C. botulinum*, is a complex consisting of the neurotoxin and a hemagglutinin. Since at least 1000 times more neurotoxin than neurotoxin-hemagglutinin complex is required to cause food poisoning in animals, it is believed that the hemagglutinin protects the neurotoxin from stomach acids and enzymes. Neurotoxin alone is rapidly inactivated by gastric enzymes and lowered pH. The complete toxin is sensitive to heat. To give you some idea of the toxicity of *C. botulinum* toxin, less than 1×10^{-9} gm of toxin can kill a mouse. Its toxicity is similar to that of the neurotoxin produced by *C. tetani*. For comparison, the lethal concentration of diphtheria toxin is 1.5×10^{-6} gm; for *C. perfringens* toxin, 3.6×10^{-6} gm.

PATHOGENESIS. Botulism results from the ingestion of contaminated food and is considered an intoxication rather than an infection. Once the active toxin is released in the intestine, it is absorbed and transported into the lymph and then the bloodstream. Clinical symptoms usually appear between 24 and 72 hours after the toxin has been absorbed from the intestinal tract. The toxin acts specifically on the peripheral nervous system, particularly the cranial nerves, and not the central nervous system. It prevents the release of acetylcholine at the neural synapses. Symptoms of intoxication include dou-

Fig. 21-4. Sporulating cells of *Clostridium botulinum* type A. Spores are subterminal. (Courtesy P. D. Walker.)

ble vision (diplopia) and dizziness, often followed by difficulty in swallowing and breathing. Nausea and vomiting may also be present during these manifestations. Death is usually due to respiratory failure. Fatality rates are high for type A poisoning (75 percent), lower for the other types (20 percent). The severity of type A intoxication may be related to the greater affinity of type A neurotoxin for nerve fibers. (See boxed essay, p. 495.)

Botulism may also be caused by the production of toxin by *C. botulinum* in wounds. Approximately 25 cases of wound botulism have been recorded in the United States, and no doubt many others go unrecognized. The symptoms are the same as those in food poisoning caused by *C. botulinum*. Type A is usually implicated, with a case fatality rate of 25 percent. Although microorganisms are present in the wound, chemotherapy with antibiotics has not proved efficacious, probably on account of lack of blood supply in necrotic areas.

Infant botulism has been termed an infection because the spores are ingested and in the intestine germinate and produce toxin. *C. botulinum* has been isolated from the feces of infants with this condition. Since everyone ingests a large number of spores each day with vegetables, fruit, and other foods, it is difficult to understand why only some infants are affected. Host susceptibility may play a predominant role. Most of the infants affected are less than 6 months old. The first signs of infection are constipation, followed by weakened sucking and swallowing and diminished gag reflex. Later there is a loss of head control. The most important aspect in the treatment of infant botulism is supportive care because of the possibility of respiratory insufficiency. The effect of antibiotics and antitoxin has not been established. Infant botulism is currently being investigated as one of the causes of sudden infant death syndrome.

LABORATORY DIAGNOSIS. Initial diagnosis depends on the identification of the toxin in the serum or stools of the affected individual. This is often followed by intraperitoneal injections of extracts of the patient's serum or liquefied stool samples into laboratory mice. The mice will succumb in 1–4 days if *C. botulinum* toxin is present in the specimens. If the suspect food is available, it too can be checked for the presence of toxin or anaerobic sporeformers (Fig. 21-4).

TREATMENT AND PREVENTION. The earlier botulism is detected, the better the prognosis. Case fatality rates in the 1950s were near 60 percent but have since declined to less than 15 percent because of advances in acute respiratory intensive care. Even before a bacteriological diagnosis is made, trivalent antitoxin, prepared against the toxins of types A, B, and E, is given intravenously. Because the antitoxin is prepared from horse serum, the patient is generally tested for sensitivity before injection. If the patient has neurological symptoms, he or she should be placed in an intensive care unit where cardiac and respiratory function may be monitored. Guanidine hydrochloride is sometimes used during treatment to enhance the release of acetylcholine in the nerve junctions to prevent respiratory failure.

BOTULISM AND COMMERCIAL POTPIE—CALIFORNIA

On August 3, 1982, a 56-year-old woman residing in Los Angeles County, California, developed diplopia, weakness, difficulty breathing, and chest pain. She had respiratory arrest on admission to the hospital but was intubated, resuscitated, and placed in intensive care. She had a history of seizure disorder, diabetes mellitus, and organic brain syndrome. An infectious disease consultant thought her subsequent fever was due to pneumonia secondary to aspiration, and he suspected botulism as the underlying cause of her illness.

The patient lives with her husband and grown son who both prepare meals for her and attempt a strict diet in consideration of her diabetes.

The son had prepared the potpie for an early evening meal. The frozen pie was baked in an oven for 40–45 minutes. As he was about to serve it to his mother, his father came home with some freshly cooked hamburgers just purchased at a takeout restaurant. The potpie was put aside on an unrefrigerated shelf. Two and one-half days later, the son came home and found his mother had just consumed this potpie without reheating it.

An uneaten portion of the potpie, still in its metal plate, was retrieved by the family members. Type A botulism toxin was found in this pie by a mouse-inoculation test performed at a U.S. Department of Agriculture laboratory in Beltsville, Maryland, and type A toxin was also demonstrated in the patient's serum by the state's Microbial Disease Laboratory.

In this situation, it is suspected that the original baking killed competing organisms in the pie and eliminated much of the oxygen. The heat-resistant, anaerobic *Clostridium botulinum*, which was evidently present and can be found in many fresh, frozen, and other food products, was then presumably able to germinate and produce toxin under the crust during storage at warm, incubator-like temperatures. Products such as potpies should be kept frozen before heating and ideally should be served hot after the first cooking. If any such products are to be saved, they should be quickly refrigerated, then reheated to hot temperatures. This would minimize any risk of botulinal poisoning.

From *MMWR* 32:39, 1983.

Most outbreaks of botulism from home-processed foods are due to improper cooking of low-acid vegetables or to allowing fish or game to putrefy at room temperature. Tomatoes have heretofore been considered high enough in acid content (pH 4.5) so that the processor could omit cooking under pressure to destroy *C. botulinum* spores. However, many varieties of tomatoes today are low in acid, and the addition of citric acid or other organic acids is now being advised for home and industrial canning. Since the *C. botulinum* toxin is heat labile, it is suggested that whenever possible

home-canned fruits or vegetables be heated to 100°C for 10 minutes before ingestion.

Gas Gangrene

The species most commonly implicated in gas gangrene is *C. perfringens*; next come *C. septicum, C. histolyticum*, and *C. novyi*. All are motile except *C. perfingens*, which is the only species that produces a capsule. This group of microorganisms can be found as indigenous members of the intestinal tract of humans and animals and as saprophytes in the soil. None of the species are invasive, but all produce potent toxins.

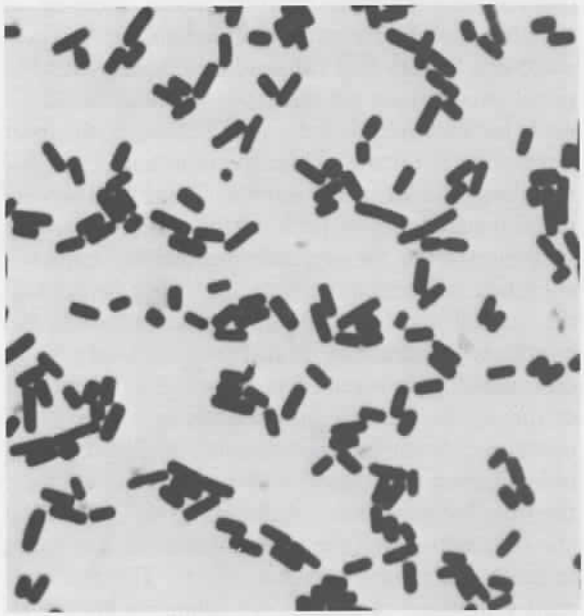
CLOSTRIDIUM PERFRINGENS TOXINS. Of the eleven toxins produced by *C. perfringens*, four are used to separate the species into five toxigenic groups (A, B, C, D, and E). Only types A and C produce disease in humans. All five types produce various proportions of four toxins (alpha, beta, epsilon, and iota), but the most important is the *alpha toxin*. Alpha toxin acts as a lecithinase and hydrolyzes lecithin, a component of eukaryotic membranes, and during infection destroys red blood cells and leukocytes. Other toxins released during infection that may take part in pathogenesis are collagenase, hemolysin, proteinase, and deoxyribonuclease. The toxin demonstrating collagenase activity is believed to attack healthy connective tissue in the skin and the connective tissue supporting muscle fibers. It is the probable cause of the extensive muscle destruction observed in gas gangrene. Hemolysin lyses the red blood cells of most mammalian species. Proteinase is thought to be important in degrading necrotic tissue, while deoxyribonuclease may destroy leukocytes by its ability to depolymerize DNA.

PATHOGENESIS. Gas gangrene is an infection that requires a site for germination of spores and replication of vegetative cells. Such a site is available when tissue has been traumatized by foreign bodies (for example, puncture wounds) and is devitalized, thereby offering an anaerobic environment. Clostridial wound infection may be divided into two major clinical conditions: *anaerobic cellulitis* and *anaerobic myositis* (true gas gangrene). Anaerobic cellulitis involves necrotic tissue, but the organism does not invade healthy tissue. Anaerobic cellulitis is the less severe infection. There is seldom toxemia or shock. True gas gangrene is often a mixed infection involving several *Clostridium* species. Most cases of the disease appear during war or after automobile accidents or surgical procedures such as those involving the uterus. Deep wounds and areas of dead tissue favor germination. Toxins are liberated by vegetative cells, and during the course of infection gases such as hydrogen and carbon dioxide are released by metabolizing bacteria. The first symptoms of gas gangrene are usually observed 72 hours after traumatization of tissue. Severe local pain is felt in the area of the wound. A few hours later there is swelling of the wound, giving it a stretched and reddened appearance. Still later the skin ruptures, revealing a necrotic, foul-smelling wound. The most important symptoms of gas gangrene are the delirium, apathy, and disorientation exhibited by the patient in the early stages of the disease. Death is believed to be due to severe toxemia and to the invasion of vital organs by the toxin.

LABORATORY DIAGNOSIS. A tentative diagnosis of gas gangrene is often made by examination of the wound for gram-positive sporeformers. This is usually difficult because *C. perfringens* does not often sporulate in tissue. In addition, the wound may contain a wide variety of other microorganisms including nonpathogenic clostridia. Presumptive identification of *C. perfringens* can be made by first inoculating a blood (rabbit, sheep, or human) agar plate with the specimen and incubating it anaerobically. Gram-stained cells show a typical "boxcar" shape as opposed to the rounded ends of other clostridial species (Fig. 21-5). Colonies of *C. perfringens* appear surrounded by an inner zone of complete hemolysis and an outer zone of incomplete hemolysis. The isolated colonies may also be subcultured to egg yolk agar plates to demonstrate the production of type A toxin (lecithinase).

TREATMENT AND PREVENTION. Successful treatment of gas gangrene consists in the immediate debride-

Fig. 21-5. Photomicrograph of *Clostridium perfringens*. Note the boxcar shape of the cells. (Courtesy P. D. Walker.)



ment of the wound to remove all dead tissue, application of antiserum that has been prepared from filtrates of the major clostridial species that produce gas gangrene, and antibiotic therapy. Penicillin and tetracyclines have been the antibiotic drugs of choice.

Treatment of gas gangrene patients with high concentrations of oxygen at elevated pressures is receiving a great deal of attention today. This procedure, called *hyperbaric oxygen therapy*, at one time was used almost exclusively for persons suffering from carbon monoxide poisoning and decompression sickness. The patient inhales 100% oxygen through an aviator's mask in a chamber initially pressurized to approximately 3 atmospheres of air for a period of 1 hour. Then follows a 30-minute period of 100% oxygen at 2 atmospheres of pressure, and finally comes a 30-minute period of 100% oxygen at 1 atmosphere of pressure. The treatment is repeated every 8 hours, the total number of treatments seldom exceeding eight. The principle behind this treatment is that anaerobes in the presence of oxygen produce a strong oxidizing agent, hydrogen peroxide, which can be lethal to the organisms. Oxygen has no effect on the circulating toxin, which must be detoxified in the body. Hyperbaric oxygen treatment is potentially very dangerous and must be administered by qualified personnel.

Gas gangrene is always a possible hazard when surgery is being performed on areas of the body that are easily contaminated by one's intestinal microflora—the lower limbs, thighs, and buttocks. Infections have been greatly reduced when the area to be operated on and surrounding tissue are first swabbed with compresses of iodophors. Best results are obtained when the area is swabbed just 15 minutes before surgery.

*Food Poisoning (*Clostridium perfringens*)*

C. perfringens is a common cause of food poisoning in humans. The condition is the result of ingestion of a large number of viable vegetative cells. Foodborne disease in the United States is due to type A *C. perfringens* and is most frequently associated with a meat product. The organism survives cooking by sporulation and then germinates when the food is cooled. If the food is not reheated, many vegetative cells are produced. Ingestion of approximately 10^8 cells will cause food poisoning. The ingested bacteria sporulate in the intestine and produce an enterotoxin that is part of the spore coat. The enterotoxin causes secretion of fluid and electrolytes (sodium and chloride ions).

Foodborne disease caused by *C. perfringens* type C is associated with a high mortality. The illness is referred to as *enteritis necroticans* and is characterized by hemorrhaging and gangrene of the intestine. The severity of the disease is believed to be due to a beta toxin that is normally inactivated by proteolytic enzymes. The disease is observed in areas outside the United States where poor nutrition results in decreased activity of proteolytic enzymes in the bowel.

C. perfringens type A food poisoning is second to staphylococcal food poisoning in number of outbreaks. Over 90 outbreaks involving 5255 individu-

als were recorded between 1976 and 1981 in the United States. The vehicles implicated in disease, in order of importance, were beef, Mexican food, turkey, ham, and chicken. Improper storage or holding temperature and inadequate cooking of food were the principal causes of disease. The major symptoms were abdominal pain, diarrhea, nausea, and vomiting. The incubation period is 24–30 hours and rarely are fatalities encountered.

Treatment of *C. perfringens* food poisoning does not require antibiotics since the disease is mild and self-limiting. It is important that body fluids and electrolytes be replenished. This type of food poisoning can be prevented by properly cooking food and, if food is to be reheated, by ensuring that the internal temperature of the food is greater than 75°C so that vegetative cells are destroyed.

Tetanus

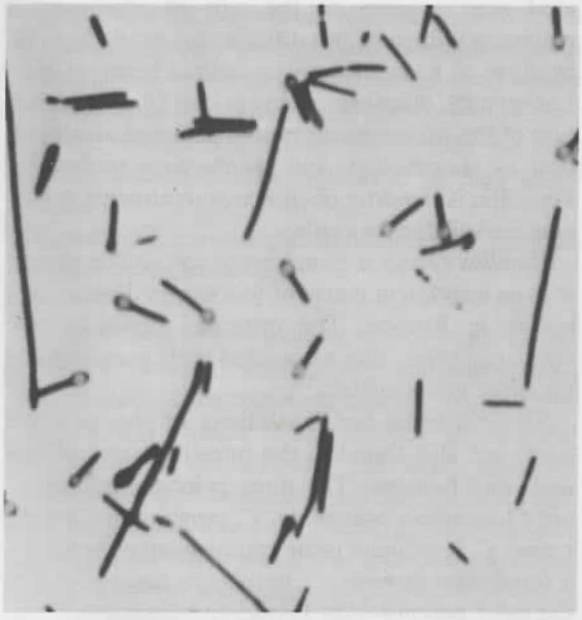
Tetanus, like gas gangrene, is an infection often stemming from a complication of traumatized tissue. The causative agent, *C. tetani*, is found primarily in cultivated soil but also appears in the intestinal tract of about 25 percent of humans. In underdeveloped countries tetanus is a common cause of neonatal death. In the United States approximately 200 cases of tetanus are reported each year.

TETANUS TOXINS. The complications of tetanus are due to the release of a potent toxin called *tetanospasmin*. Tetanospasmin is a heat-labile protein with a toxicity similar to that of *C. botulinum* toxin. Every milligram of toxin-nitrogen is lethal for 2×10^8 laboratory mice. The toxin (it is a neurotoxin) is released upon lysis of the vegetative cell. It acts on the central nervous system, and its binding capacity is dependent on the number of sialic acid residues in the ganglioside. Its mode of action, like that of botulinum toxin, is blocking the transmission of nerve impulses. Unlike *C. botulinum* toxin, however, tetanus toxin produces spastic paralysis because of its action on the central nervous system; *C. botulinum* toxin affects the peripheral nerves. *C. tetani* also produces a hemolysin that can be reversibly inactivated by oxygen.

PATHOGENESIS. *C. tetani* gains entrance to the body through wounds or abrasions. The spores germinate only in the absence of oxygen, and such a condition is encountered when the wound is deep or contains considerable necrotic tissue. Piercing of the ears, circumcisions, and abortions are also predisposing factors that can lead to tetanus when hygienic precautions are not taken. *Tetanus neonatorum*, for example, is a frequent cause of death in developing countries. This type of tetanus is the direct result of cutting the umbilical cord with unsterilized instruments or packing the umbilical stump with mud. Once the organism germinates in the tissue, the toxin acts directly on the motor neurons of the central nervous system. The toxin causes the continued contraction of various voluntary muscles, which is referred to as *tetany*. The symptoms of the disease appear in 4–6 days as a rule; however, up to 6 weeks is not unusual. This wide range in incubation time seems to depend on two factors: the time for anaerobic conditions to develop at the site of infection and the time required for any toxin to reach the central nervous system. The predominant symptoms of tetanus are primarily muscle rigidity and muscle spasm. Rigidity affects the muscles of the jaw (masseter muscles), abdomen, and spine. Muscle spasm is seen most often in the mouth but may occur in any part of the body. The convulsive contraction of the voluntary muscles of the jaw is referred to as *lockjaw* (*trismus*). Death is often the result of some type of respiratory failure—pneumonia, pulmonary edema, or asphyxia.

LABORATORY DIAGNOSIS. Diagnosis can be made on clinical grounds. Patients usually have a wound, exhibit trismus, and have a history of no immunization. Stained smears of the material from a wound are very unreliable for the identification of *C. tetani* because of the presence of other clostridia. Material from the wound can be cultured directly on blood agar or transferred to cooked meat medium heated to 80°C for 10 to 15 minutes to destroy nonsporeformers. After 24 hours incubation on blood agar, the plate should be examined for swarming (motility). A spore stain reveals charac-

Fig. 21-6. Photomicrograph of *Clostridium tetani* demonstrating the characteristic tennis racket–shaped terminal spores. (Courtesy P. D. Walker.)



teristic terminal spores shaped like tennis rackets (Fig. 21-6). Definitive proof that the isolated species is *C. tetani* is obtained by determining neurotoxin production. This is measured by injecting the filtrate from a pure culture of *C. tetani* into the leg of a mouse; a second mouse receives an injection of the filtrate plus the tetanus antitoxin. After a few hours the unprotected mouse displays stiffness and muscular spasms in the injected leg that extend to the tail and then to the opposite leg. The protected mouse shows no adverse effects from the toxin.

TREATMENT. Suspected cases of tetanus, even before isolation of *C. tetani*, are first treated by administration of tetanus antitoxin to neutralize any toxin in the blood. The antitoxin will not neutralize toxin already fixed to nerve cells. Antibiotics such as penicillin are usually given to destroy any clostridial cells and prevent further toxin production. Where there is a foul contaminated wound, it is important to remove surgically the necrotic tissue or debris that is creating the anaerobic environment. Even if all these measures are performed, once the toxin is attached to the nerve cells they will not alter the course of the disease. The basic treatment is symptomatic; that is, the convulsions and spasms must be controlled by drugs. Barbiturates are therefore routinely employed to sedate the patient and to control the spasm.

A great deal of controversy had been centered around the use of antitoxin prepared from horse serum (tetanus immune globulin, or TIG). Some physicians will not administer such antitoxin because many individuals are sensitive to the horse serum proteins. It is also believed that the body's immune system eliminates the injected antitoxin too rapidly, and thus it provides inadequate protection. For these reasons some hospitals and private practitioners use only antitoxin prepared from the gamma globulin fraction of humans.

PREVENTION. The only effective way to control tetanus is through the prophylactic use of the tetanus toxoid. The toxoid, which incorporates an adjuvant (aluminum salts) to increase its immunizing potency, is given routinely with diphtheria toxoid and pertussis vaccine (DTP). An initial dose is normally administered a few months after birth, a second dose 4 to 6 months later, and finally a reinforcing dose 6 to 12 months after the second injection. A final booster is given between the ages of 4 and 6 years. For many years booster doses of tetanus toxoid were administered every 3 to 5 years; that practice has been discontinued since it has been shown that a single booster dose can afford protection for 10 to 20 years. Serious hypersensitivity reactions have occurred when too many doses of toxoid were administered over a period of years. Booster doses today are generally given only when an individual has sustained a wound infection.

Other Clostridial Conditions

Antibiotic-associated *pseudomembranous colitis* is a condition occurring after treatment with antibiotics such as lincomycin, clindamycin, and ampicillin. *Clostridium difficile* has been implicated as the

agent of this type of colitis in humans. This organism is infrequently found in the intestinal tract and, when present, appears in small numbers. The normal microbial flora is believed to prevent the growth of *C. difficile*, but antibiotic administration disrupts the normal flora and presumably permits growth of this clostridial species. Experiments with animal models (Syrian hamster) have shown an increase of *C. difficile* up to 10^8 cells per gram of cecal content after administration of ampicillin. The disease is believed to be toxin mediated since the *C. difficile* produces two toxins, A and B, both of which are cytotoxic and lethal to animals. Studies have also demonstrated that *C. difficile*, isolated from patients who developed pseudomembranous colitis during ampicillin therapy, is sensitive to ampicillin. It is believed that a beta-lactamase induced in the cecal flora after ampicillin therapy permits sensitive *C. difficile* to resist the antibiotic.

Psuedomembranous colitis can occur during or after the termination of antimicrobial therapy and may persist for months. Initial symptoms include diarrhea, fever, and nausea, which may progress to dehydration, loss of electrolytes, septicemia, shock, and death. Vancomycin administered orally can prevent disease since the microbial agent is sensitive to the drug.

Necrotizing enterocolitis is a fulminating, sometimes lethal condition of sick premature babies. Symptoms include abdominal distention, blood in stools, shock, and gas in the bowel wall. Breast-fed babies are protected against necrotizing enterocolitis. It is hypothesized that immunoglobulins or other factors in the mother's milk protect the baby from the overgrowth of clostridial species in the intestinal tract.

SUMMARY

The bacilli are aerobic sporeformers; the clostridia, anaerobic sporeformers. The Bacilli pathogenic to humans are *Bacillus anthracis* and *B. cereus*. *B. anthracis*, a microorganism found in the soil, is the causative agent of anthrax, a disease of animals. Most human disease results from contact with animal hides or hair. The most common form of anthrax is cutaneous anthrax, in which a large ulcerated area appears at the site of infection. A respiratory form of the disease can result from inhalation of spores found on animal hides or hair. Laboratory diagnosis relies primarily on cultivation of the microorganism on media and identification by morphology and biochemical properties. Penicillin is the drug of choice in treatment. A vaccine is available for cattle.

Bacillus cereus is found in the soil and on plants. It is an infrequent cause of foodborne disease, primarily in Europe. The organism produces two types of toxins, one associated with vomiting and the other with diarrhea.

The clostridia are inhabitants of the soil, but some are also found in the intestinal tract of animals and humans. The three principal pathogens are *Clostridium botulinum*, *C. perfringens*, and *C. tetani*. *C. botulinum* is the causal agent of botulism, a foodborne disease. *C. botulinum* toxin is one of the most potent toxins known to humans and may cause death. Botulism symptoms can also result from wound contamination by *C. botulinum* or by ingestion of spores of the organism by infants. Diagnosis of botulism is based on toxin identification in stools or serum. Treatment requires the use of trivalent antitoxin. Botulism can be prevented in the home by sterilization of foods before canning.

C. perfringens is a cause of gas gangrene and food poisoning. Gas gangrene results from contamination of a wound with the organism and the organism's ability to produce a potent toxin that can cause death. Food poisoning results from ingestion of vegetative cells, which sporulate in the intestinal tract and release an enterotoxin. The enterotoxin does not cause fatalities. Gas gangrene can be treated by debridement of the wound and application of antiserum plus antibiotic therapy. Placing a patient in a hyperbaric chamber at elevated concentrations of oxygen can also be used for more severe cases.

C. tetani is the causal agent of tetanus, a disease resulting from soil contamination of a wound. The

toxin produced is as potent as the botulinal toxin and works on the nervous system. The disease is seldom observed in the United States because the toxoid is part of the DTP vaccine-toxoid complex that is administered to children a few months after birth.

QUESTIONS FOR STUDY

1. How can one distinguish between the major pathogenic clostridia?
2. Is anthrax primarily a disease of humans? How can humans contract anthrax?
3. What kind of capsule is produced by *Bacillus anthracis*?
4. Where are *B. anthracis* and *B. cereus* normally found?
5. What is the major virulence factor (or factors) of *C. perfringens*?
6. What is the mechanism of action of *C. botulinum* toxin in the host? Tetanus toxin?
7. What clostridial species is believed to be associated with pseudomembranous colitis? What are the circumstances in which this disease occurs?
8. What is the treatment for gas gangrene? Tetanus?
9. What is the difference in the symptoms of food poisoning caused by *C. perfringens* and *C. botulinum*?
10. What is the virulence determinant for *B. anthracis*?

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22. *CORYNEBACTERIUM DIPHTHERIAE*

OBJECTIVES

To describe the symptoms associated with diphtheria
To describe the mechanism of action of diphtheria toxin
To describe the in vivo test for determining the toxigenicity of *C. diphtheriae*
To describe those methods used to treat and prevent diphtheria
To outline the schedule for immunization against diphtheria
To explain the importance of diphtheroids and *Listeria* as agents of disease

General Characteristics
Pathogenesis
Immunity
Laboratory Diagnosis
Epidemiology
Treatment and Prevention
Other Corynebacteria and *Listeria*

Diphtheria was first described as a clinical entity in the early 1800s. The causative agent, *Corynebacterium diphtheriae*, however, was not isolated until the late 1880s. At that time Loeffler and Klebs described the microorganism in morphological terms as a rod-shaped cell. In 1888 it was discovered that the pathogenicity of the diphtheria bacillus was related to its ability to produce a potent exotoxin. When sterile culture filtrates were injected into certain laboratory animals, they reproduced all the features of the human disease. In 1890 Emil Behring found that guinea pigs injected subcutaneously with diphtheria cultures acquired a specific immunity and that the blood of these test animals exhibited toxin-destroying properties. In 1894 immune serum from sheep was used in Germany for the treatment of diphtheria. In 1920 a mass immunization program against diphtheria was begun in the United States, reducing the attack rate from 151 cases per 100,000 in 1920 to less than 0.5 per 100,000 in 1958. In 1983 there were only 5 reported cases of diphtheria in the United States. The effect that the immunization program has had on incidence and mortality ratios is illustrated in Figure 22-1. Today, in most of the United States, diphtheria is a clinical rarity.

GENERAL CHARACTERISTICS

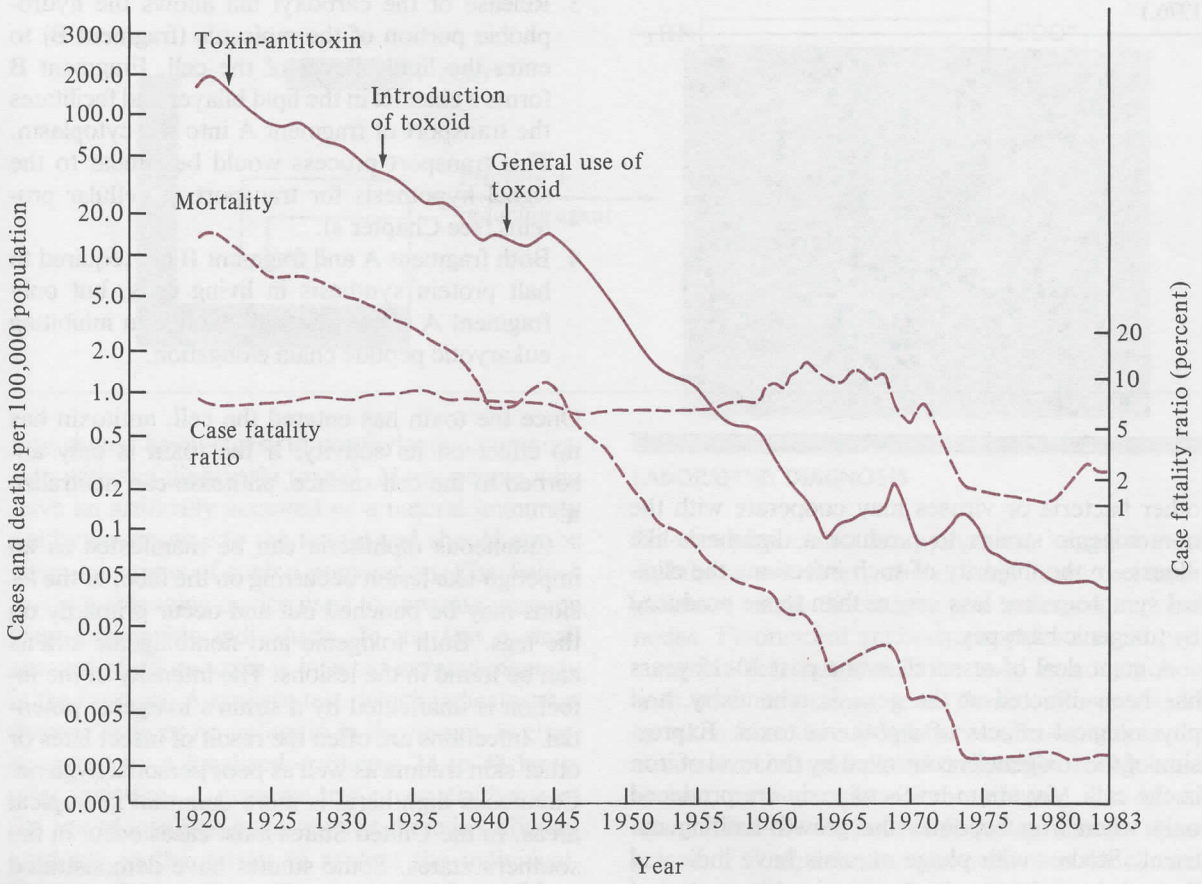
Corynebacterium diphtheriae is an obligate aerobe found in the upper respiratory tract of humans. It is a nonmotile, gram-positive pleomorphic bacillus averaging between 2 and 6 μm in length and 0.5 and 1.0 μm in diameter. In older cultures the bacilli appear club shaped. The bacilli are capable of concentrating phosphate in the form of polymerized metaphosphate granules. When the cell is stained with methylene blue, the metaphosphate granules become apparent and give the bacillus a beaded appearance (Fig. 22-2). These granules are called *volutin, Babès-Ernst bodies*, or *metachromatic granules*. Because of their unusual arrangement after cell division the bacilli appear in palisades (parallel rows) and resemble Chinese letters.

There are three characteristic biotypes of *C. diphtheriae: gravis, intermedius*, and *mitis*. Their names purportedly correspond to the severity of the disease; however, this is not always the case. The toxigenicity of these strains is directly related to the presence of a temperate (does not lyse the cell) bacteriophage that carries information for toxin production (tox gene). If "cured" of its temperate virus, the bacterium is no longer toxigenic. Many normally nontoxigenic strains of corynebacteria can be converted to toxigenic strains by infecting them with the appropriate temperate bacteriophage.

PATHOGENESIS

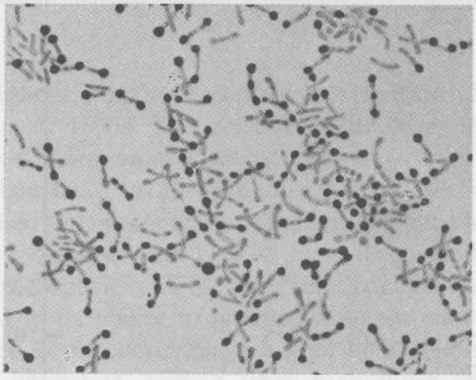
Diphtheria may actually be divided into two types, respiratory and cutaneous. The incubation period for respiratory or faucial diphtheria is 2–6 days. Symptoms initially include sore throat, slight fever, and malaise. The bacilli are harbored in the mucous membranes of the passageways of the oral cavity. The toxin elaborated by the bacilli causes necrosis of the mucosal cells, which aids in bacterial multiplication. The bacilli do not invade any underlying tissue, nor do they enter the bloodstream. The in-

Fig. 22-1. Diphtheria annual incidence and mortality ratios and case fatality ratios in the United States, 1920–1980. (From Centers for Disease Control, Atlanta, GA.)



flammatory response of the infected tissue causes the accumulation of a grayish white exudate that clots and attaches to various passageways forming a pseudomembrane. The pseudomembrane consists of neutrophils, corynebacteria, and desquamated epithelial cells enmeshed in a dense fibrinous network. The membrane can be found over the mucosa of the mouth, pharynx, larynx, or trachea. If the inflammatory response involves the laryngeal or tracheal areas, respiratory obstruction can occur. If not remedied by tracheostomy or intubation, this condition can cause suffocation. Death in most instances results from heart failure owing to the effects of the toxin on heart tissue or from asphyxiation. Nontoxigenic strains of gravis, intermedius, and mitis have been isolated from patients with clinical symptoms similar to those of diphtheria. In these patients a sufficient level of antitoxin was still present in the serum for protection against toxigenic strains. It has been suggested that

Fig. 22-2. Stained smear of diphtheria bacilli. The darkly stained areas are polyphosphate or metachromatic granules. (From R. R. Gillies and T. C. Dodds, *Bacteriology Illustrated*. London: Churchill Livingston, 1976.)



other bacteria or viruses may coopérate with the nontoxigenic strains to produce a diphtheria-like illness. In the majority of such infections the clinical symptoms are less severe than those produced by toxigenic biotypes.

A great deal of research in the past 10–15 years has been directed at the genesis, chemistry, and physiological effects of diphtheria toxin. Expression of the tox gene is controlled by the level of iron in the cell. Maximum levels of toxin are produced only when iron becomes the growth-limiting nutrient. Studies with phage mutants have indicated that a bacterial factor is also involved in control of toxin production. It is currently believed that *C. diphtheriae* synthesizes an aporepressor molecule that in the presence of iron becomes activated and represses the synthesis of toxin. Toxin is released by the bacterial cell as a single inactive polypeptide but is later cleaved into two fragments, A and B. Toxin is believed to enter eukaryotic cells and inhibit protein synthesis in the following way:

1. The hydrophilic (carboxyl) end of the toxin is positively charged and interacts with specific receptors on the cytoplasmic membrane of the eukaryotic cell.
2. A cellular protease nicks the polypeptide, causing the release of the carboxyl tail (Fig. 22-3), and also affects the amino end of the polypeptide.
3. Release of the carboxyl tail allows the hydrophobic portion of the molecule (fragment B) to enter the lipid bilayer of the cell. Fragment B forms a channel in the lipid bilayer and facilitates the transport of fragment A into the cytoplasm. This transport process would be similar to the signal hypothesis for transport of cellular proteins (see Chapter 4).
4. Both fragment A and fragment B are required to halt protein synthesis in living cells, but only fragment A is enzymatically active in inhibiting eukaryotic peptide chain elongation.

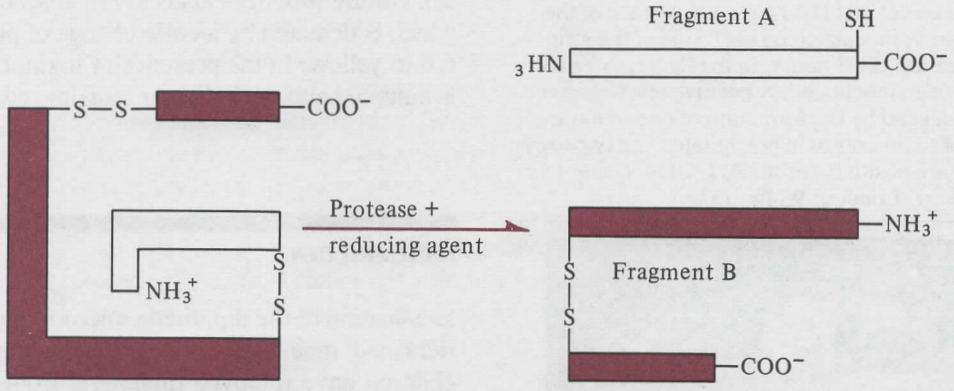
Once the toxin has entered the cell, antitoxin has no effect on its activity. If the toxin is only absorbed to the cell surface, antitoxin can neutralize it.

Cutaneous diphtheria can be manifested as an impetigo-like lesion occurring on the face, or the lesions may be punched out and occur primarily on the legs. Both toxigenic and nontoxigenic strains can be found in the lesions. The intensity of the infection is unaffected by a strain's toxigenic potential. Infections are often the result of insect bites or other skin trauma as well as poor personal hygiene. Cutaneous diphtheria is more common in tropical areas. In the United States most cases occur in the southern states. Some studies have demonstrated that the strain carried in the cutaneous lesion is also carried in the respiratory tract in more than 50 percent of the cases. It has been suggested, but not proved, that the cutaneous carrier, particularly in crowded living conditions, is a source of respiratory diphtheria.

IMMUNITY

Because diphtheria is much less severe in the fully immunized individual, all members of the commu-

Fig. 22-3. Proposed action of cellular protease on the polypeptide toxin of *Corynebacterium diphtheriae*.

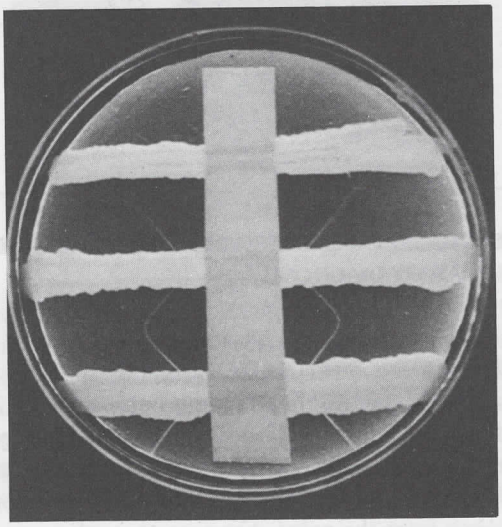


nity should have access to prophylactic immunization with the diphtheria toxoid. Many people who have an artifically acquired or a natural immunity are hypersensitive to the toxoid and should not be given injections of such a preparation. The *Schick test* is a screening device used to separate immune from susceptible individuals. In the test a small amount of dilute toxin is injected intracutaneously in the forearm. A positive test, which indicates that there is little or no antitoxin in the serum, is characterized by a localized erythema 24 to 48 hours after injection. A negative test or no erythema at the site of injection indicates that there is sufficient antitoxin in the serum to protect the individual. False-positives often arise among older children and adults despite high levels of antitoxin in their serum. For this reason a control test is performed on the opposite arm using toxin detoxified by heating. An allergic response, however, to the detoxified protein can still occur. If the tissue response at the control site parallels that at the test site in size and duration, the Schick test is considered negative. Hypersensitivity can also be determined by means of a variant of the Schick test called the *Moloney test*, in which subcutaneous injections of the toxoid are made and the inflammatory response, if any, is measured. These cutaneous tests are no longer performed with any regularity.

LABORATORY DIAGNOSIS

To the physician, the symptoms suggestive of diphtheria are fever, chills, sore throat, serosanguineous nasal discharge, and enlarged cervical nodes. Fluorescent antibody provides a rapid presumptive test but should be used in conjunction with other laboratory tests. Swab cultures should be taken from the nares, pharynx, nasopharynx, or cutaneous sites and plated on Loeffler (coagulated serum) medium, potassium tellurite, and blood agar. After 24–48 hours all plates should show growth if *C. diphtheriae* was present in the clinical specimen. Smears are made of the isolates and Gram-stained to be sure the organism is a gram-positive rod. The morphology of *Corynebacterium* species is determined by the growth medium. Slide specimens prepared from Loeffler's medium will reveal metachromatic granules. On tellurite agar, gravis biotypes produce large, gray, flat, dry colonies with irregular edges; mitis biotypes produce a black, convex, and moist colony; intermedius biotypes are very small, flat, and gray to black. The blood agar is used to detect any beta-hemolytic streptococci that might be the cause of the respiratory infection. The isolates can also be used for in vivo guinea pig inoculation or in vitro gel diffusion

Fig. 22-4. Gel diffusion plate for demonstrating the toxigenicity of strains of *Corynebacterium diphtheriae* (Elek method). A filter strip soaked in diphtheria antitoxin is placed in the center and just below the surface of the agar. The plate is inoculated on each side of the strip and incubated for 24–48 hours. In the figure, three test strains have been inoculated. A positive test (toxigenic strain) is evidenced by the formation of one or more white lines of toxin-antitoxin precipitate. The two lower test cultures are positive. (From R. J. Olds, *Color Atlas of Microbiology*. London: Wolfe, 1975.)



tests to determine the toxigenicity of the isolated strain. In the in vivo test a small area of the abdomen of two guinea pigs is shaved. One animal is administered diphtheria antitoxin intraperitoneally. After 2–4 hours both animals are injected intradermally with a dilution of the unknown suspension of bacilli. If toxigenic bacilli were present in the unknown suspension, only the animal unprotected by antitoxin will sustain any ill effects. The animal protected by antitoxin will show no adverse effects. In the in vitro test (*Elek method*) a sterile strip of filter paper impregnated with diphtheria antitoxin is pushed below the surface of a molten enriched agar medium. After the agar hardens, an inoculum of the unknown culture is streaked at right angles to the filter strip. If the unknown bacilli are toxigenic, a precipitate, indicating specific antigen-antibody interaction, will be formed (see Fig. 22-4).

An additional test for toxin production involves the use of Chinese hamster ovary cell cultures. The cell culture produces acids in the absence of toxin, which is detected by a color change of phenol from red to yellow. In the presence of toxin, cell growth is inhibited and the indicator remains red.

EPIDEMIOLOGY

Eradication of the diphtheria microorganism in carriers and maintenance of immunization levels in children have removed diphtheria from the list of major infectious diseases in the United States. Epidemics do occur periodically, but they are limited to very small geographical areas. Less than 3 percent of all the counties in the United States between 1969 and 1980 recorded cases of diphtheria. Most vulnerable to diphtheria are people of low socioeconomic status with limited access to health care facilities. More than 80 percent of the recorded cases of diphtheria occur in the 1- to 19-year-old age group, with the highest incidence among those between 10 and 14 years of age. (See boxed essay, p. 509.) Disease is also frequent in older adults because of low immunization status. Complications after infection are severe in older adults.

TREATMENT AND PREVENTION

In any suspected cases of diphtheria the antitoxin should be administered to neutralize preformed toxin. The antitoxin is still prepared from horse serum, and approximately 10 percent of persons receiving therapy develop allergic reactions. The physician should therefore determine the patient's sensitivity to the serum before administration of the antitoxin. Antibiotics, although not a substitute for the antitoxin, will destroy the toxin-producing bacilli. Penicillin and erythromycin are effective in

FATAL DIPHTHERIA—WISCONSIN

A fatal case of diphtheria was recently reported to the Wisconsin State Department of Health and Social Services. A 9-year-old unimmunized girl developed listlessness and a sore throat on June 30, 1982, 10 days after arriving at a camp in Colorado operated by a religious group that does not accept immunizations. On July 3, she returned to Wisconsin on a camp bus along with other unimmunized children and adults who had also attended the camp. On July 6, a physician evaluated the patient for her sore throat; a throat culture was taken and oral penicillin prescribed. The patient was hospitalized on July 8 for persistent sore throat, diminished fluid intake, and gingival bleeding. The throat culture obtained July 6 was reported to contain normal flora, group A beta-hemolytic streptococci, and large numbers of diphtheroids. The patient was transferred on July 8 to a tertiary-care children's hospital.

On admission, she was afebrile and had moderate upper airway obstruction, diffuse ecchymoses, bleeding from the nose and gums, prominent cervical adenopathy, and swelling of the jaw and throat. Initially, the pharynx was poorly visualized due to trismus. On later examination, it revealed severe hemorrhagic and necrotic tonsillitis; no membrane was observed. Treatment with penicillin G, gentamicin, moxalactam, peritoneal dialysis, and platelet transfusions was instituted. The hospital course was complicated by disseminated intravascular coagulation, cardiac conduction abnormalities, and mental confusion. The patient died on July 14. A *Corynebacterium* species isolated from a throat culture obtained July 10 was subsequently confirmed by the Milwaukee Bureau of Laboratories and State Laboratory of Hygiene to be a toxigenic strain of *C. diphtheriae*.

An investigation was undertaken to determine the source of exposure to *C. diphtheriae* and to identify and examine the patient's contacts. The camp session had been attended by 108 employees, campers, and counselors from Wisconsin and 12 other states; many were unimmunized. In addition, 119 immediate and extended family members and hospital employees in Wisconsin, who might have had close contact with the patient after onset of illness, were identified. With the aid of state and local health departments and private physicians, 224 of the 227 contacts were examined. None reported respiratory illness before or after exposure to the patient, and nasopharyngeal or throat cultures obtained from 218 contacts were negative for *C. diphtheriae*.

This is the first reported diphtheria-associated fatality in Wisconsin since 1968; none has been reported in Colorado since 1976. Nationally, the number of reported diphtheria cases declined steadily from 435 in 1970 to 5 in 1981.

The absence of any isolates of *C. diphtheriae* among the numerous contacts cultured in this investigation may be, in part, a result of delay in diagnosing the case and, therefore, delay in obtaining cultures.

From *MMWR* 31:553, 1982.

destroying the bacilli. Erythromycin is especially valuable in the eradication of the carrier state.

For cutaneous diphtheria, compresses soaked in penicillin can be applied to the lesions.

Mass immunization has been a major factor in the control of diphtheria. It is generally recommended that the diphtheria toxoid be mixed with tetanus toxoid and pertussis vaccine (DTP) and administered as early as 3 months after birth, followed by second and third injections at 6- to 8-week intervals. A booster dose is recommended before entry into school. Immunity persists for nearly 10 years after full immunization. For unimmunized persons 7 years and older a series of three doses of tetanus and diphtheria toxoid (*Td*) is administered. Pertussis vaccine is not administered because pertussis is infrequent and less severe in this age group. Diphtheria prophylaxis is recommended for household contacts of patients with respiratory diphtheria—especially those previously unimmunized or inadequately immunized. The contacts should receive diphtheria toxoid as well as an intramuscular injection of penicillin G benzathine. The major problem in diphtheria control is elimination of the carrier state. Present control efforts include (1) isolation and treatment of clinical cases, (2) use of erythromycin and penicillin in treatment of household and other contacts shown to be carriers of *C. diphtheriae*, and (3) full immunization of all preschool children with diphtheria toxoid and maintenance of diphtheria antitoxin serum levels by booster injections.

OTHER CORYNEBACTERIA AND *LISTERIA*

Many species of *Corynebacterium* are found in humans and animals. Some have been implicated in human infection, while others are considered nonpathogens. The nonpathogenic corynebacteria, which are collectively called *diphtheroids*, reside on the skin and in the mouth, vagina, and urethra. The anaerobic corynebacteria have now been reclassified in the genus *Propionibacterium*. For example, *C. acnes* is now *P. acnes*. The diphtheroids are pleomorphic, nonmotile, catalase-positive, gram-positive bacilli. Most produce metachromatic granules and exhibit the typical palisade arrangement found in smears of *C. diphtheriae*.

Although not primary pathogens, the diphtheroids are opportunistic. They are known to be the cause of endocarditis in cases in which the patient has been compromised by cardiac surgery or valvular prostheses. Diphtheroids have also been implicated in meningitis and osteomyelitis. Since diphtheroids do not elaborate exotoxin, the toxigenicity test is critical in determining the presence of *C. diphtheriae*.

Bacteriological diagnosis of diphtheroid infections is difficult because of the confusion with strains of *Streptococcus*, which under certain growth conditions appear rod shaped, and with *Listeria monocytogenes*, a motile, gram-positive diphtheroid bacillus that is also catalase positive. Isolation of diphtheroids can be achieved by using a selective medium containing a base of trypticase soy and yeast extract supplemented with polysorbate 80 (Tween 80) to support the growth of lipophilic strains, oil red to distinguish lipophilic from nonlipophilic, and Furoxone to inhibit growth of gram-negative bacilli and gram-positive cocci. Differentiation of *Corynebacterium* species is routinely performed through biochemical reactions. Characteristics of some of these species are listed in Table 22-1.

L. monocytogenes is a diphtheroid-like bacillus found primarily in the intestinal tract of birds and mammals. It sometimes infects humans; the disease is called *listeriosis*. Infections are more common in neonates, the microorganisms being transferred from mother to fetus. The microorganisms have been associated with stillbirth and meningitis. Adult patients compromised by underlying illness are more prone to infection. Infection may also occur in persons who are in contact with animal fetuses. Recent evidence has demonstrated that food-borne transmission is important in sporadic outbreaks. Certain cultural characteristics of *L. monocytogenes* provide a means of identification as well as differentiation from diphtheroids:

Table 22-1. Characteristics of Species of *Corynebacterium* That Cause Disease in Humans

Species	Normal habitat	Disease	Important biochemical properties
C. diphtheriae	Normal flora of upper respiratory tract of humans	Respiratory (diphtheria), cutaneous	Ferments glucose and maltose, produces catalase; most strains reduce nitrate
C. equi	Found in soil near livestock	Pulmonary infection in patients with neoplastic disease	Cannot ferment carbohydrates
C. xerosis	Normal flora of skin and nasopharynx of humans	Endocarditis in patients with prosthetic cardiac valves	Can reduce nitrate, ferments several carbohydrates
C. pseudodiphtheriticum (C. hofmannii)	Normal flora of pharynx of humans	Endocarditis	Cannot ferment carbohydrates, produces urease, can reduce nitrate
Group JK	? May be indigenous to skin	Blood, tissue, and wound infections in those with indwelling catheters	Cannot produce urease, cannot reduce nitrate, cannot ferment carbohydrates
C. ulcerans	? Pharynx of humans	Diphtheria-like illness	Produces urease, does not reduce nitrate
C. pseudotuberculosis (C. ovis)	Respiratory tract of domestic animals	Lymphadenitis, pneumonia	—

1. Clinical specimens from blood or cerebrospinal fluid can be inoculated onto sheep blood agar plates. Colonies of *L. monocytogenes* are surrounded by a narrow zone of beta hemolysis. If isolated on tryptose agar, the colonies appear green when observed with light reflected obliquely through them.
2. Isolates of *L. monocytogenes* can be inoculated into two tubes of semisolid motility medium, one at room temperature (20–25°C) and the other at 35°C, for 2–5 days. At room temperature the microorganism produces an "umbrella" growth below the surface of the medium. Most other diphtheroids are nonmotile and do not grow below the surface of the motility medium. Motility at 35°C is minimal.
3. Pathogenic strains of *L monocytogenes* may be differentiated from nonpathogenic strains by an ocular test. A drop from an overnight culture is introduced into the conjunctival sac of a rabbit. Purulent conjunctivitis develops in a few days and then heals.

Treatment of listeriosis includes administration of antibiotics such as ampicillin and tetracycline.

SUMMARY

C. diphtheriae is the causal agent of diphtheria. The organism is a gram-positive aerobic bacillus in which polyphosphate granules are synthesized. The pathogenic species carry a bacteriophage that carries the determinant for toxin production. The diphtheria toxin is the virulence factor and is a protein composed of two subunits. The toxin is transported into the cytoplasm of the host cell where it inhibits protein synthesis. Diphtheria may be of two types, respiratory and cutaneous. Only the respiratory disease is a major cause of fatalities. Death from diphtheria is usually the result of as-

phyxiation owing to blockage of the bronchioles or heart failure owing to the effects of toxin. Diphtheria is diagnosed by fluorescent antibody in conjunction with biochemical tests and animal inoculation. Treatment of diphtheria during the course of disease is accomplished by the administration of antiserum. Antibiotics eradicate the bacteria but have no effect on toxin. Few cases of diphtheria are observed in the United States since the advent of mass immunization with diphtheria toxoid, which is part of the complex including pertussis vaccine and tetanus toxoid.

A number of nondiphtherial corynebacteria called diphtheroids are found on the skin and in the mouth, vagina, and urethra. Diphtheroids become important pathogens particularly in those compromised by underlying conditions or disease. Diphtheroids may be confused with *Listeria monocytogenes* but can be differentiated from them by biochemical tests.

QUESTIONS FOR STUDY

1. Where is the site of infection by *C. diphtheriae*? Can the organism invade the bloodstream?
2. What are the conditions required for toxin production by *C. diphtheriae*?
3. What are the immediate causes of death from infection by *C. diphtheriae*?
4. Describe the mechanism of diphtheria toxin uptake by the host cell and the toxin's effects on host metabolism.
5. How is diphtheria toxin detected in the laboratory?
6. What are diphtheroids, and where are they found? What is their importance in human disease?
7. How is *C. diphtheriae* detected by culture techniques?
8. What is the immunosupportive management for diphtheria?
9. What antibacterial agent or agents are employed to treat diphtheria?

ADDITIONAL READINGS

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Jellard, C. H. Toxigenic and nontoxigenic *Corynebacterium diphtheriae*. *Lancet* I:650, 1980.

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23. GRAM-NEGATIVE ENTERIC BACILLI

OBJECTIVES

To list the important traits of the principal members of the Enterobacteriaceae

To briefly outline in a flowchart the laboratory procedures used to isolate and differentiate members of the Enterobacteriaceae

To describe the intestinal and extraintestinal diseases caused by *Escherichia coli*

To describe the virulence factors associated with infections caused by *E. coli, Salmonella* species, *Shigella* species and *Yersinia* species

To explain the methods of treatment for intestinal and extraintestinal disease caused by the Enterobacteriaceae

To describe the different disease states associated with salmonella infections

To describe the epidemiology of plague

To list the opportunistic Enterobacteriaceae and the types of infections with which they are associated

Characteristics of the Enterobacteriaceae
Isolation of the Enterobacteriaceae
Selective Enrichment Media
Primary Isolation Media
Differential Selective Media
Screening and Identification of the Enterobacteriaceae
Escherichia coli
Pathogenesis
Extraintestinal Disease
Intestinal Disease
Enterotoxigenic *E. coli*
Enteropathogenic *E. coli*
Enteroinvasive *E. coli*
Laboratory Diagnosis
Indole Test
Methyl Red Test
Voges-Proskauer Test
Citrate Test
Treatment
Salmonella Species
Pathogenesis
Gastroenteritis
Enteric Fever (Typhoid, Paratyhphoid, and Nontyphoidal Fevers)
Bacteremia
Carrier State
Epidemiology
Laboratory Diagnosis
Treatment
Prevention and Control
Shigella Species
Pathogenesis
Epidemiology
Laboratory Diagnosis
Treatment
Prevention and Control
Yersinia Species
Yersinia pestis
Pathogenesis
Epidemiology
Laboratory Diagnosis
Treatment
Prevention and Control
Yersinia enterocolitica and *Y. pseudotuberculosis*
Providencia and *Arizona* Species
Enterobacter Species
Proteus Species
Klebsiella Species
Serratia marcescens

Many gram-negative nonsporeforming bacilli inhabit the intestinal tract of humans and animals. These enteric bacilli for the most part belong to the family Enterobacteriaceae (Table 23-1).* Other gram-negative bacilli that are either found as intestinal pathogens or are recovered from infections associated with enteric bacilli belong to the families Vibrionaceae and Pseudomonadaceae. They will be discussed in subsequent chapters.

The term *coliform* has sometimes been used to denote all the enteric bacilli. It is used more often, however, to describe gram-negative, fermentative inhabitants of the intestinal tract: *Escherichia coli, Klebsiella pneumoniae*, and *Enterobacter aerogenes*.

In the past 20 years the overuse of antibiotics has caused a dramatic increase in the number of hospital-associated (nosocomial) diseases caused by enteric bacilli. As a group the enteric bacilli have supplanted the gram-positive microorganisms, such as the staphylococci and streptococci, as the major cause of disease in the hospital. Although many enteric species are commensals, patients compromised by underlying illnesses and immunosuppressive drugs are subject to life-threatening diseases by these microorganisms.

*A great deal of confusion has arisen from the classification of the Enterobacteriaceae. The Edwards and Ewing scheme had predominated for many years until the Bergey's classification was proposed. Both are presented in Table 23-1 so that certain genera will be recognized during the discussion of infectious agents.

Table 23-1. Classification of the Enterobacteriaceae

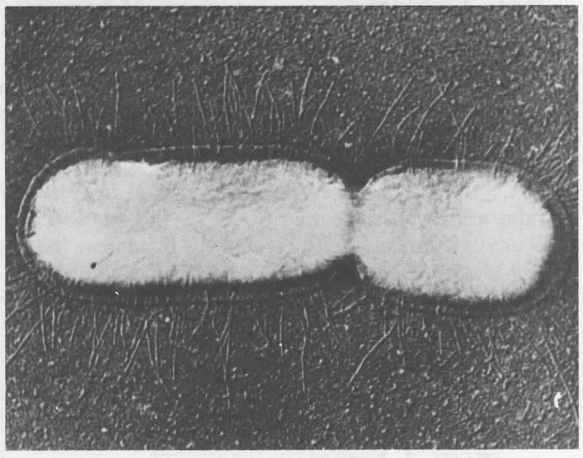
Edwards and Ewing	*Bergey's Manual* (8th edition)
FAMILY. Enterobacteriaceae TRIBE I. Escherichieae GENUS I. *Escherichia* SPECIES. *E. coli* GENUS II. *Shigella* SPECIES. *S. dysenteriae* *S. flexneri* *S. boydii* *S. sonnei* TRIBE II. Edwardsielleae GENUS I. *Edwardsiella* SPECIES. *E. tarda* TRIBE III. Salmonelleae GENUS I. *Salmonella* SPECIES. *S. choleraesuis* *S. typhi* *S. enteritidis* GENUS II. *Arizona* SPECIES. *A. hinshawii* GENUS III. *Citrobacter* SPECIES. *C. amalonaticus* *C. freundii* *C. diversus* TRIBE IV. Klebsielleae GENUS I. *Klebsiella* SPECIES. *K. pneumoniae* *K. oxytoca* *K. ozaenae* *K. rhinoscleromatis* GENUS II. *Enterobacter* SPECIES. *E. cloacae* *E. sakazakii* *E. aerogenes* *E. agglomerans* GENUS III. *Hafnia* SPECIES. *H. alvei* GENUS IV. *Serratia* SPECIES. *S. marcescens* *S. liquefaciens* *S. rubidaea* *S. plymuthica* *S. odorifera* *S. fonticola* TRIBE V. Proteeae GENUS I. *Proteus* SPECIES. *P. vulgaris* *P. mirabilis* GENUS II. *Providencia* SPECIES. *P. stuartii* *P. rettgeri* *P. alcalifaciens* GENUS III. *Morganella* SPECIES. *M. morganii* TRIBE VI. Yersineae GENUS I. *Yersinia* SPECIES. *Y. enterocolitica* *Y. pseudotuberculosis* *Y. pestis* *Y. intermedia* *Y. frederiksenii* *Y. ruckeri* TRIBE VII. Erwinieae (plant pathogens) GENUS I. *Erwinia* GENUS II. *Pectobacterium*	FAMILY. Enterobacteriaceae GENUS I. *Escherichia* SPECIES. *E. coli* GENUS II. *Edwardsiella* SPECIES. *E. tarda* GENUS III. *Citrobacter* SPECIES. *C. freundii* *C. intermedius* GENUS IV. *Salmonella* SPECIES. *S. choleraesuis* *S. typhi* *S. enteritidis* *S. arizonae* GENUS V. *Shigella* SPECIES. *S. dysenteriae* *S. flexneri* *S. boydii* *S. sonnei* GENUS VI. *Klebsiella* SPECIES. *K. pneumoniae* *K. oxytoca* *K. ozaenae* *K. rhinoscleromatis* GENUS VII. *Enterobacter* SPECIES. *E. cloacae* *E. aerogenes* GENUS VIII. *Hafnia* SPECIES. *H. alvei* GENUS IX. *Serratia* SPECIES. *S. marcescens* GENUS X. *Proteus* SPECIES. *P. vulgaris* *P. mirabilis* *P. morganii* *P. rettgeri* *P. inconstans* GENUS XI. *Yersinia* SPECIES. *Y. enterocolitica* *Y. pseudotuberculosis* *Y. pestis* GENUS XII. *Erwinia* (primarily plant pathogens) SPECIES. *E. herbicola* (considered a human pathogen)

CHARACTERISTICS OF THE ENTEROBACTERIACEAE

No single morphological or biochemical characteristic can be used to differentiate all of the Enterobacteriaceae. However, some generalizations can be made about the majority of them.

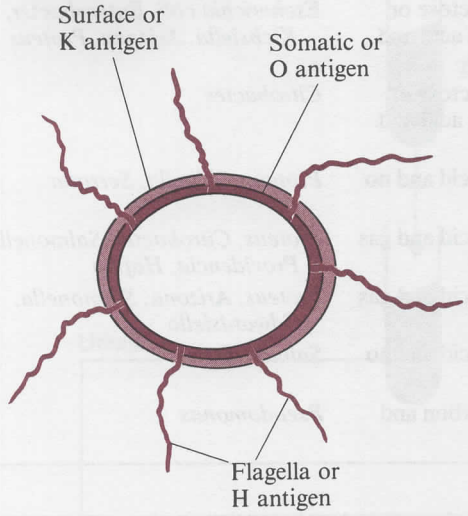
1. All members of the family Enterobacteriaceae are gram-negative aerobic or facultatively anaerobic rods varying in length from 1 to 8 μm. They ferment glucose, do not produce oxidase, and reduce nitrates to nitrites.
2. Most can be cultivated on ordinary laboratory media and stained with aniline dyes.
3. Most species are active fermenters of glucose and other carbohydrates. Many of the pathogens can be separated from the nonpathogens on the basis of the former's inability to ferment lactose.
4. Motile as well as nonmotile species are found in the group. Those that are motile are peritrichous (have flagella over their entire surface).
5. Pili are found on the cell surface of members of the Enterobacteriaceae (Fig. 23-1). The sex pili are important in the conjugation process, particularly for the transfer of antibiotic resistance factors (R factors) between the same or different family members. The transfer of R factors is believed to take place infrequently in the human gut unless the individual is being treated with antibiotics. The more numerous adhesive pili found on the surface of the organisms are responsible for the adherence of some species to epithelial surfaces. These antigens are protein and are also called K antigens, but they are to be distinguished from the capsular K antigens, which are polysaccharide.
6. Because of the ability of members of the Enterobacteriaceae to transfer antibiotic resistance plasmids, antibiotic susceptibility testing must be routinely performed on clinical isolates before an antibiotic regimen can be instituted. Some clinical isolates have shown resistance to one to ten different major therapeutic agents.

Fig. 23-1. Electron micrograph showing the presence of pili on the surface of a dividing gram-negative bacterial cell. (From J. P. Duguid and R. R. Gillies, *J. Pathol.* 74:397, 1957.)



7. The lipopolysaccharides of the cell wall of the enteric bacilli are toxic and are called *endotoxins*. As yet no direct relationship has been established between pathogenicity and the endotoxin of the enteric microorganisms. The biological properties of the endotoxins are discussed in Chapter 12.
8. Many enteric species are capable of producing toxic substances that are bactericidal to related species of bacteria but not themselves. These toxic compounds are called *bacteriocins*. Bacteriocins are discussed in Chapter 4.
9. The Enterobacteriaceae are classified by their cell envelope–associated antigens, that is, the O, K, and H antigens (Fig. 23-2). The *O* or *somatic antigen* is the O-specific polysaccharide of the lipopolysaccharide (LPS) component of the cell wall. LPS has three regions: the O-specific polysaccharide, a core polysaccharide, and lipid A. The O polysaccharide is responsible for the O antigen specificity of the Enterobacteriaceae. The various O antigens contain different sugar side chains that make possible the differentiation of species. When the O-spe-

Fig. 23-2. The antigenic components found on the surface of members of the Enterobacteriaceae.



cific side chains are present, the bacteria are referred to as smooth (S forms). When the O-specific side chains are absent, the bacteria are referred to as rough (R forms). (*Smooth* and *rough* refer to the colonial morphology on agar media. R forms, however, may appear smooth if they contain a capsule.) The O-specific polysaccharide protects the cell against host resistance factors. Certain O antigens seem to be associated with certain diseases. R forms are more easily phagocytized and are less virulent than S forms. The core oligosaccharide consists of components that may be similar or different in a given group of bacteria. All *Salmonella*, for example, share a common core, but four different cores have been found in *E. coli* strains. Lipid A is the toxic component of the LPS complex. It is similar in all of the different Enterobacteriaceae. The capsular polysaccharide of the Enterobacteriaceae is referred to as the *K antigen*. Among the *E. coli* strains there are 100 different K antigens. Not all species in the family possess K antigens, but those that do are usually more pathogenic, probably because of their ability to resist phagocytosis. Certain K antigenic types are also more frequently isolated from certain infections than other K antigenic types. The reason is not clear. Flagellar protein antigens are referred to as *H antigens*. They are responsible for motility. Their identification often represents the last step in the procedure of serotyping various strains within a genus of bacteria. (Serotypes are divisions based on antigenic relatedness, for example, similarity of O antigens. Biotypes are strains of the same serotype that differ in a biochemical characteristic, for example, sugar fermentation.)

10. Because of the ease of cultivating them, many Enterobacteriaceae have been used in research efforts, especially in the elucidation of metabolic pathways, protein synthesis, and genetic mechanisms. *E. coli*, for example, is being currently used in experiments involving genetic recombination (see Chapter 5).

ISOLATION OF THE ENTEROBACTERIACEAE

The enteric laboratory represents the largest division in the clinical microbiology section of a hospital. Enteric bacilli are involved in numerous hospital-associated diseases. They can be isolated from feces, urine, blood, and cerebrospinal fluid. The kind of medium to use for isolation depends on the source of the specimen. If it is from a nonintestinal source such as the blood or urine, the organisms may be a practically pure culture because human blood and urine are usually sterile. Hence the specimen may be plated on blood agar and a differential medium. If the specimen is from an intestinal source, the infecting pathogen will be greatly outnumbered by commensal species and will initially require a selective enrichment medium. A variety of selective, differential, and enrichment media are available to resolve these problems.

Table 23-2. Key to Identification of the Enterobacteriaceae on Triple Sugar Iron Agar

Reaction	Biochemical interpretation	Organisms most likely to be involved
Acid slant, acid-gas butt, no H_2S produced	Glucose fermented and lactose or sucrose fermented with acid and gas produced	*Escherichia coli, Enterobacter, Klebsiella, Arizona, Proteus*
Acid slant, acid-gas butt, H_2S produced	Glucose fermented and lactose or sucrose fermented with acid and gas produced	*Citrobacter*
Alkaline slant, acid butt, no H_2S produced	Glucose fermented with acid and no gas produced	*Proteus, Shigella, Serratia*
Alkaline slant, acid-gas butt, no H_2S produced	Glucose fermented with acid and gas produced	*Proteus, Citrobacter, Salmonella, Providencia, Hafnia*
Alkaline slant, acid-gas butt, H_2S produced	Glucose fermented with acid and gas produced	*Proteus, Arizona, Salmonella, Edwardsiella*
Alkaline slant, acid butt, H_2S produced	Glucose fermented with acid and no gas produced	*Salmonella*
No reaction, but green pigment produced	Peptone only source of carbon and energy	*Pseudomonas*

SELECTIVE ENRICHMENT MEDIA

If the infecting pathogen (for example, *Salmonella* or *Shigella* species) is from an intestinal source, a selective medium is often needed. Selective media such as tetrathionate, Hajna GN broth, and selenite broth inhibit gram-positive bacteria and coliforms, permitting the rapid multiplication of *Salmonella* and *Shigella* species. Once the pathogen has been grown to sufficient numbers, it can be inoculated into various selective or differential media.

PRIMARY ISOLATION MEDIA

Primary isolation media include eosin–methylene blue (EMB) and MacConkey agar. These media contain chemicals that inhibit gram-positive organisms. They also contain lactose, a disaccharide that permits a presumptive differentiation of lactose fermenters from non–lactose fermenters. Lactose fermenters such as *E. coli* and *Enterobacter aerogenes* produce colored colonies, while the non–lactose fermenters such as *Salmonella* and *Shigella* exhibit colorless colonies because they do not ferment lactose.

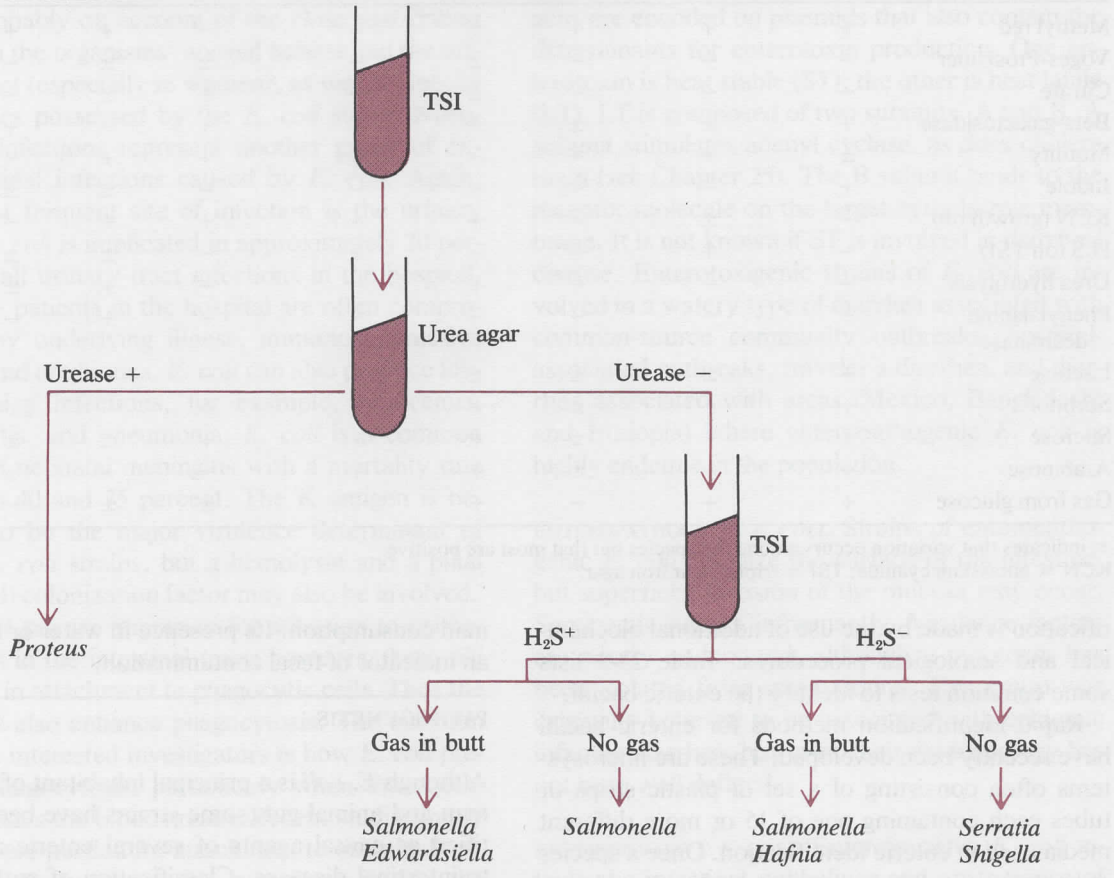
DIFFERENTIAL SELECTIVE MEDIA

Differential selective media such as *Salmonella-Shigella* (SS), deoxycholate-citrate, bismuth sulfite, and brilliant green agars are used primarily for the isolation of *Salmonella* and *Shigella* species. These media are selective against gram-positive organisms as well as most coliforms.

SCREENING AND IDENTIFICATION OF THE ENTEROBACTERIACEAE

Many biochemical tests can be used to identify a member of the Enterobacteriaceae. A few tests, however, have been found that lead to identification of more than 90 percent of the clinical isolates. If the isolate is from a fecal sample and was unable to ferment lactose, it can be presumptively identified by streaking on triple sugar iron agar, lysine iron agar, and urea agar. Reactions on these media permit the presumptive identification of species of *Arizona, Salmonella,* and *Shigella* and of *Yersinia enterocolitica*—usually involved in infections of the intestinal tract. If the specimen is from a nonin-

Fig. 23-3. Separation of the Enterobacteriaceae on urea agar. Triple sugar iron (*TSI*) agar serves as the culture source for separation on urea agar.



testinal source, it can be inoculated into the same media used for fecal samples plus citrate agar, ornithine motility medium, and indole medium. Reactions on these media permit the presumptive identification of enteric bacilli that are routinely involved in infections outside the intestinal tract: *E. coli, Edwardsiella* species, *Klebsiella* species, *Citrobacter* species, and others. The reaction on triple sugar iron agar is the most important means of separating the various genera of Enterobacteriaceae.

An isolated colony from the selective or differential media is first stabbed into the butt of a triple sugar iron (TSI) agar slant, then streaked onto the surface of the slant. The TSI agar tube contains glucose (0.1%), sucrose (1%), and lactose (1%), plus phenol red to indicate fermentation and ferrous sulfate to detect hydrogen sulfide production. The small amount of glucose in the TSI agar slant, as compared to the other carbohydrates, enables one to detect the fermentation of this sugar. If glucose is the only carbohydrate fermented, the small amount of acid produced on the slant is oxidized, and the slant remains alkaline (red). Acid produced in the butt is not oxidized, and the butt remains yellow. All inoculated TSI tubes are read after 18–24 hours (Table 23-2). The combination of TSI and urea agar reactions can be a useful tool for separating many important genera (Fig. 23-3). Final iden-

Table 23-3. Biochemical Tests Used to Identify Some of the Major Genera of Enterobacteriaceae

Test or substrate	*Escherichia*	*Salmonella*	*Shigella*	*Klebsiella*	*Enterobacter*	*Serratia*	*Proteus*	*Yersinia*
Methyl red	+	+	+	±	−	−	+	+
Voges-Proskauer	−	−	−	±	+	±	±	−
Citrate	−	+	−	±	+	+	±	−
Beta-galactosidase	+	±	±	+	+	+	−	+
Motility	±	+	−	−	+	+	+	−
Indole	+	−	−	−	−	−	±	±
KCN (growth on)	−	±	−	+	+	+	+	−
H_2S (on TSI)	−	+	−	−	−	−	±	±
Urea hydrolysis	−	−	−	+	−	−	+	±
Phenylalanine deaminase	−	−	−	−	−	−	+	−
Lactose	+	±	±	±	+	−	−	−
Sorbitol	+	+	±	+	+	+	−	±
Sucrose	±	−	±	+	+	+	±	±
Arabinose	+	+	±	+	+	−	−	+
Gas from glucose	+	+	−	+	+	±	±	−

± indicates that variation occurs among the species but that most are positive.
KCN = potassium cyanide; TSI = triple sugar iron agar.

tification is made by the use of additional biochemical and serological procedures. Table 23-3 lists some common tests to identify the enteric bacilli.

Rapid identification methods for enteric bacilli have recently been developed. These are microsystems often consisting of a set of plastic strips or tubes each containing one of 15 or more different media used in enteric identification. Once a species of bacteria is isolated, a small inoculum is added to each of the media, incubated, and read in 12–24 hours. Up to 95 percent correlation has been found between results obtained with the microsystems and those produced by the conventional methods.

ESCHERICHIA COLI

E. coli is a permanent resident of the intestinal tract of humans and animals and is not a primary pathogen. One gram of feces contains from 1×10^7 to 1×10^8 *E. coli*. Because of its abundance in the intestinal tract and its ease of cultivation, *E. coli* is used as an index of the suitability of water for human consumption. Its presence in water or foods is an indicator of fecal contamination.

PATHOGENESIS

Although *E. coli* is a principal inhabitant of the human and animal gut, some strains have been identified as causal agents of several enteric and extraintestinal diseases. Classification of pathogenic *E. coli* isolates according to serotypes has been difficult since there are over 160 O antigens, 100 K antigens and 55 H antigens. Some of these surface antigens are important adhesins that enable the organism to attach to epithelial cells. The majority of pathogenic as well as nonpathogenic *E. coli* strains possess a common type of pilus (some current literature still uses the word fimbria) called *type 1*. The eukaryotic cell receptor for type 1 is mannose. Other types of pili appear to be found exclusively on enteropathogenic strains of *E. coli*.

Extraintestinal Disease

Extraintestinal disease caused by *E. coli* is usually the result of person-to-person contact. The organ-

ism may also travel from the intestine to the urinary tract. In the community outside the hospital *E. coli* is the most common cause of urinary tract infection, probably on account of the close association between the organisms' normal habitat and the urinary tract (especially in women), as well as special properties possessed by the *E. coli* strain. Nosocomial infections represent another group of extraintestinal infections caused by *E. coli*. Again, the most frequent site of infection is the urinary tract. *E. coli* is implicated in approximately 20 percent of all urinary tract infections in the hospital. Because patients in the hospital are often compromised by underlying illness, immunosuppressive drugs, and antibiotics, *E. coli* can also produce life-threatening infections, for example, septicemia, meningitis, and pneumonia. *E. coli* is a common cause of neonatal meningitis with a mortality rate between 40 and 75 percent. The K antigen is believed to be the major virulence determinant of these *E. coli* strains, but a hemolysin and a pilial (fimbrial) colonization factor may also be involved.

Type 1 pili are important for adhesion to epithelial cells in the intestinal tract; however, these pili also aid in attachment to phagocytic cells. Thus the pili may also enhance phagocytosis. The question that has interested investigators is how *E. coli* prevents phagocytosis, particularly when the organism invades the bloodstream. Recent studies imply that pili are needed for attachment to intestinal epithelial cells but once the organisms invade the bloodstream pili are not produced (phase variation). Instead the capsule (K antigen) provides the organism with a means of inhibiting phagocytosis.

Intestinal Disease

There are three major groups of *E. coli* strains associated with intestinal illness: *enterotoxigenic, enteropathogenic*, and *enteroinvasive*.*

ENTEROTOXIGENIC *E. COLI*. Enterotoxigenic strains of *E. coli* are characterized by their ability to colonize the small intestine and by the production of one or both of two types of enterotoxin. Colonization is associated with a specific type of pilus called *colonization factor antigen (CFA)*. These surface antigens are encoded on plasmids that also contain the determinants for enterotoxin production. One enterotoxin is heat stable (ST); the other is heat labile (LT). LT is composed of two subunits, A and B. A subunit stimulates adenyl cyclase, as does cholera toxin (see Chapter 25). The B subunit binds to the receptor molecule on the target cytoplasmic membrane. It is not known if ST is involved in diarrheal disease. Enterotoxigenic strains of *E. coli* are involved in a watery type of diarrhea associated with common-source community outbreaks, hospital-associated outbreaks, traveler's diarrhea, and diarrhea associated with areas (Mexico, Bangladesh, and Ethiopia) where enteropathogenic *E. coli* is highly endemic in the population.

ENTEROPATHOGENIC *E. COLI*. Strains of enteropathogenic *E. coli* colonize the surface of the intestine, but superficial invasion of the mucosa may occur. Septicemia occurs infrequently. Virulence factors are poorly understood, although a cytotoxin has been isolated from some strains. This group was originally believed to be associated with epidemic infantile diarrhea; however, their epidemiology has not been well defined.

ENTEROINVASIVE *E. COLI*. Enteroinvasive *E. coli* invade the intestinal epithelium and are a cause of dysentery (bloody stool). The involved serotypes are nonmotile. A toxin produced by these species resembles the cytotoxin produced by *Shigella dysenteriae*. This toxin is also believed to be produced by enteropathogenic strains of *E. coli*. The toxin may also play a role in pathogenesis, as do the LT and ST enterotoxins.

*A fourth group, recently recognized, causes a hemorrhagic colitis. Most cases result from ingestion of partially cooked hamburger.

LABORATORY DIAGNOSIS

E. coli is a lactose fermenter and is readily separated from other enteric species by its characteristic colonial appearance on EMB agar and biochemical reactions in the indole, methyl red,

Voges-Proskauer, and citrate tests. These tests are collectively called the *IMViC tests* (*i* is added for euphony, i.e., a pleasant sound when pronouncing).

Indole Test

Tryptone broth is rich in protein containing the amino acid tryptophan. If the tryptophan is metabolized by bacteria, pyruvic acid, ammonia, and indole are released. When Kovac's reagent is added to the culture medium, a red color develops if indole is present.

Methyl Red Test

A special medium called methyl red Voges-Proskauer (MR-VP) medium is inoculated with the test organism and incubated for at least 48 hours. Methyl red, which is red at pH 4.4 or below and yellow at pH 6 and above, is added to the culture tube. If the tube remains red, the test is positive, indicating that growth of the microorganisms has dropped the pH of the medium below 5. If the added methyl red turns yellow in the culture tube, the test is negative and indicates that microbial growth has not reduced the pH of the medium below 5.

Voges-Proskauer Test

MR-VP medium is inoculated with the test organism. The VP test is based on the presence or absence of acetylmethyl carbinol, which is produced by certain microorganisms such as *Enterobacter aerogenes*. In the test an alcoholic solution of alphanaphthol and KOH is added to the culture medium. A reddish color indicates the presence of acetylmethyl carbinol.

Citrate Test

Simmons' citrate agar is inoculated with the test organism and incubated 24–48 hours. Simmons' citrate agar contains $(NH_4)_3PO_4$ as a nitrogen source and citrate as the sole carbon source. If the test organism can use citrate, alkaline conditions will be created and the medium will turn from its original green to a deep Prussian blue.

Since the biochemical activities of the pathogenic species of *E. coli* are identical to those of the nonpathogenic species of the gut, serological identification is required. Serological identification is performed by slide agglutination tests.

TREATMENT

In gastrointestinal infections antibiotics are not recommended. Successful treatment depends on the replacement of water and electrolytes. Chloramphenicol, tetracyclines, and nalidixic acid are used to treat urinary tract infections. Many enteric bacilli are notoriously resistant to a wide variety of antibiotics. It is advised that isolated species be subjected to in vitro antibiotic susceptibility tests if antibiotic therapy is undertaken.

SALMONELLA SPECIES

Members of the genus *Salmonella* are rod-shaped cells that with few exceptions are actively motile. They are capable of causing disease in both animals and humans. Salmonellae are hardy microorganisms that can survive in moist environments and in the frozen state for several months. All *Salmonella* diseases are the result of ingestion of the bacilli from contaminated water, food, or fomites.

The three recognized species of *Salmonella* are *S. typhi, S. choleraesuis*, and *S. enteritidis*. *S. enteritidis* includes all the *Salmonella* organisms except those of *S. typhi* and *S. choleraesuis*. Although there are more than 1500 serotypes of *S. enteritidis*, about 70 percent of human *Salmonella* infections in the United States are caused by 10 serotypes, the most common of which is *S. enteritidis* serotype typhimurium. (Many investigators still prefer to use the serotypes of *S. enteritidis* as species names; for example, *S. enteritidis* serotype typhimurium is called *S. typhimurium*. We will follow this practice.)

PATHOGENESIS

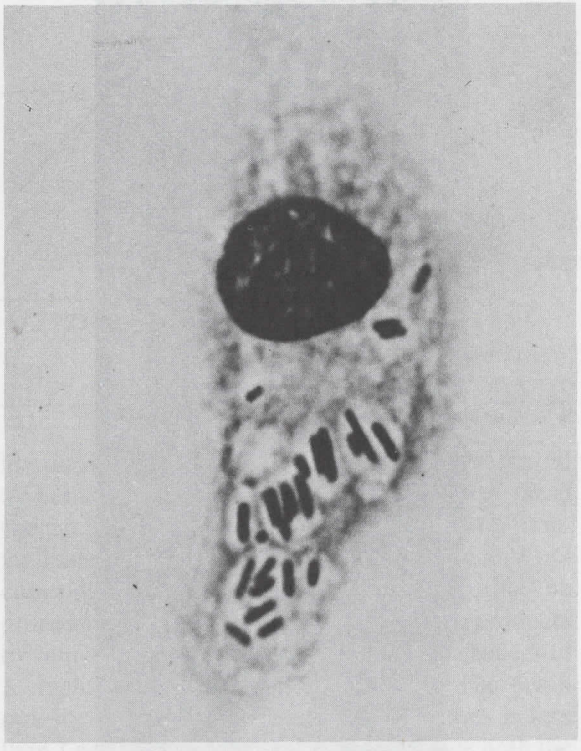
The manifestations of disease caused by the salmonellae may include gastroenteritis, enteric fever, and bacteremia, and each may be associated with the carrier state. Most infections in the United States are of the gastrointestinal variety and result from the ingestion of contaminated food or water. Enteric fevers (for example, typhoid fever) are seldom observed in the United States, although they are quite common in developing countries.

The factors that determine the severity of infection by salmonellae are size of the inoculum, the serotype, and the state of health of the host. The number of viable salmonellae that are required to cause infection after ingestion of contaminated water or food depends on the serotype and host factors. The calculated doses involved in *Salmonella* outbreaks have been determined in recent studies in which the vehicle was obtained. They have demonstrated that as few as 17 or as many as 1×10^9 microorganisms may be required to cause disease.

Gastroenteritis

When contaminated material has been ingested, the bacilli multiply in the intestinal tract. Later they penetrate the epithelial mucosal cells of the ileum and cause an inflammatory response in the lamina propria of the intestinal villi. Lymphatic barriers prevent the less virulent serotypes from invading the bloodstream, and the infection remains intestinal. Symptoms appear 4 or 5 days after ingestion of the bacilli and include diarrhea, which is not dependent on the production of an enterotoxin. The mechanism of diarrhea production is believed to be similar to that caused by *Vibrio cholerae* and pathogenic *E. coli*; that is, adenyl cyclase is induced with the formation of adenosine 3′5′-cyclic phosphate (cyclic AMP), resulting in the hypersecretion of fluid. The diarrhea lasts 3–5 days and is accompanied by fever and abdominal pain. During acute gastroenteritis 10^6 to 10^9 salmonellae per gram of feces may be present.

Fig. 23-4. Murine peritoneal macrophage (×2000 before 30% reduction) showing the presence of several *Salmonella typhimurium* cells. (From J. V. DeSiderio and S. Campbell, *J. Infect. Dis.* 148:563, 1983.)



Enteric Fever (Typhoid, Paratyphoid, and Nontyphoidal Fevers)

The ingested bacilli that cause enteric fever are more invasive than those that cause only gastroenteritis. They are capable of resisting host defenses and can multiply in the macrophage (Fig. 23-4). The primary step in pathogenesis is penetration of the intestinal mucosa. Presumably there is alteration of the mucosal surface that permits the pathogen to gain access to the epithelial cell. Once contact has been made with the epithelial surface there is localized degeneration of the brush border of the epithelial cell. The microorganism is then surrounded by the cytoplasmic membrane of the epithelial cell and is subsequently observed in a vacuole within the

Fig. 23-5. Typhoid ulceration of ileum. Ulcerations appear as small rimmed craters. (Courtesy Air Force Institute of Pathology, AFIP 2803.)

cytoplasm. Salmonellae are transported through the epithelial cells within these vacuoles. The salmonellae eventually enter the lamina propria and can enter lymph nodes where they colonize the macrophage. Organisms that have infected the lymph system can enter the circulation, from whence they can infect any tissue of the body including the intestine. The reinfection of lymph tissue in the intestine provides an avenue for shedding in the stool. The bacilli routinely infect the gallbladder, where they multiply and provide a focal point for shedding of bacilli into the intestinal tract.

The major species or serotypes that cause enteric fevers are *S. typhi* (typhoid fever), *S. paratyphi A* and *S. paratyphi B* (paratyphoid fever), and *S. schottmülleri* (nontyphoidal enteric fever). The incubation period for enteric fever is approximately 1–2 weeks. During the first week there is a gradual increase in fever accompanied by anorexia and general aches and pains. A continual headache is the principal symptom, and more than 60 percent of patients demonstrate a nonproductive cough. During the second week the fever remains as high as 104°F, and there are symptoms of diarrhea and abdominal discomfort and general weakness. During this period, which corresponds to the chronic bacteremic stage, rose-colored spots may appear on the abdomen. If no complications occur, the disease begins to terminate during the third week. Complications include abscess formation, intestinal perforation (Fig. 23-5), pneumonia, and thrombophlebitis. The death rate for typhoid fever varies from 2 to 10 percent. Paratyphoid fever is similar to typhoid fever but of shorter duration and milder.

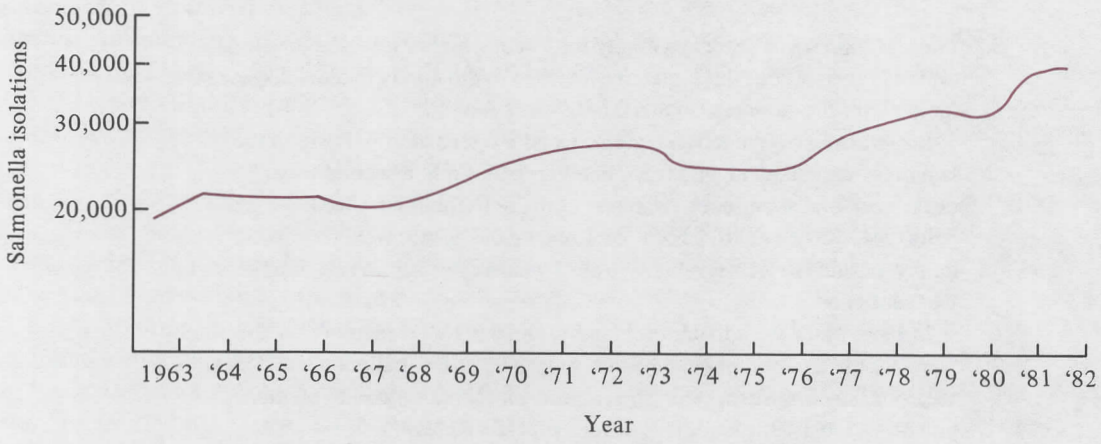
Bacteremia

Salmonellae may be transiently present in the bloodstream or may persist for long periods of time. The more invasive species such as *S. typhimurium* and *S. choleraesuis* are usually involved in sustained bacteremia. The most serious consequence of bacteremia is metastatic infection, which may involve the bones and joints, the cardiovascular system, and the meninges. Fever and chills are the symptoms associated with bacteremia.

Carrier State

It has been suggested that the asymptomatic carrier state is a more frequent consequence of *Salmonella* infection than is gastroenteritis. Shedding of salmonellae normally occurs 5 to 6 months after the development of an acute infection. Carriers, however, are classified as those who shed bacilli for 1 year after infection. The factors that appear to determine whether the carrier state will develop are (1) the species or serotype involved (*S. typhi, S. paratyphi A*, and *S. paratyphi B* are frequently as-

Fig. 23-6. Reported human *salmonella* isolations, 1963–1980. (From Centers for Disease Control, Atlanta, GA.)



sociated with the carrier state), (2) the dose of salmonellae (doses too small to cause overt disease may induce the carrier state), and (3) disorders of the biliary tract (diseases of the biliary tract are asymptomatic and serve as a source for the shedding of bacilli). Chronic carriers are most often elderly people. (See boxed essay, p. 526.)

EPIDEMIOLOGY

Over 35,000 cases of salmonellosis were reported to the Centers for Disease Control in 1982. Only 492 of these were cases of typhoid fever. The highest incidence of disease occurs among young children and the elderly. The greatest number of cases occurs in children less than 1 year old, and it is in this age group that mortality is highest. The increased susceptibility of this age group may be related to several factors. One of these factors may be the hydrochloric acid in the stomach. The stomach is a barrier to many microorganisms, including the salmonellae. Children less than 2 months of age produce little hydrochloric acid. Patients with gastrectomies or who are taking antacids are also more susceptible to infection by ingested salmonellae.

S. typhi, S. paratyphi A, and *S. paratyphi B*, unlike most other *Salmonella* species, are harbored by humans and not animals. Transmission of these species is from person to person via fecally contaminated food or water. Contamination of food occurs only by secondary contamination during the processing or handling of meat and animal products by human carriers. Often asymptomatic carriers of *S. typhi* have caused large epidemics. The death rate for typhoid fever in the United States is less than 1 percent, but in developing countries both the number of cases and the death rate are high.

Nontyphoidal *Salmonella* species can be found in a wide variety of animal species, which serve as a common source of infection to humans. The main sources are turkeys, chickens, eggs and egg products, swine, and other domestic animals. Because of the mass processing of meats and other animal-related products used for human consumption, the number of cases of salmonellosis is rising. Since 1976 there has been a steady increase in the number of cases of nontyphoidal salmonellosis (Fig. 23-6). Nontyphoidal *Salmonella* infections are spread from animal to animal and then to humans either directly or indirectly via animal-processing procedures. Person-to-person transfer seems to be less important than contaminated foodstuffs. The majority of cases of salmonellosis occur when large

TYPHOID MARY

Mary Mallon was a cook in a family for 3 years, and in 1901 she developed typhoid fever. About the same time a visitor to the family had the disease. One month later the laundress in this family was taken ill.

In 1902, Mary obtained a new place, and 2 weeks after her arrival the laundress was taken ill with typhoid fever. In a week, a second case developed, and soon seven members of the household were sick.

In 1904, Mary went to a home on Long Island. There were four in the family, besides seven servants. Within 3 weeks after her arrival, four servants were attacked.

In 1906, Mary went to another family, and 6 of the 11 members of this family were attacked with typhoid between August 27 and September 3. At this time, the cook was first suspected. She entered another family on September 21, and on October 5 the laundress developed typhoid fever.

In 1907, she entered a home in New York City and 2 months after her arrival 2 cases developed, one of which proved fatal. During these 5 years, "Typhoid Mary" is known to have been the cause of 26 cases of typhoid fever.

She was virtually imprisoned by the New York Department of Health in a hospital from March 19, 1907. Cultures taken every few days showed bacilli now and then for 3 years. Sometimes the stools contained enormous numbers of typhoid bacilli and again for days none could be found.

Typhoid Mary then escaped from observation until 1914. In October of that year, she was engaged as cook in the Sloane Hospital for Women in New York. In January and February of 1915, an outbreak of typhoid occurred, principally among the doctors, nurses, and help of the institution, involving 25 cases. The cook was suspected but she left the premises on a few hours' leave, and did not return or leave her address. She was, however, located by the health department under an assumed name, and an investigation established her identity as the famous Typhoid Mary.

A subsequent study of her career showed that she had infected still other individuals beyond those already mentioned, and that she may have given rise to the well-known waterborne outbreak of typhoid in Ithaca, New York, in 1903, involving over 1300 cases. The fact is that a person by the name of Mary Mallon had been employed as a cook in the vicinity of the place where the first case appeared, and from which contamination of the water supply occurred.

From M. J. Rosenau, *Preventive Medicine and Hygiene* (6th ed.). New York: Appleton, 1935.

groups of people are served dishes that were prepared 24–48 hours before and were perhaps left standing for several hours at room temperature. A major source of infection discovered in 1970 was pet turtles. An estimated 200,000 cases of turtle-associated salmonellosis occurred between 1970 and 1971 in the United States, primarily in children.

Salmonella infections are commonly associated with enclosed environments such as hospitals, homes for the mentally retarded, and nursing homes. These institutions also have the highest mortality. Most epidemics begin by ingestion of contaminated foodstuffs or medical products of animal origin—pancreatin, vitamins, gelatin. From these initial cases of infection the organisms may be spread by contact between persons. Person-to-person spread is particularly important in mental institutions where personal hygiene is at a minimum. Nursery epidemics are often related to the presence of an infant "shedder" of the salmonellae.

LABORATORY DIAGNOSIS

Diagnosis of *Salmonella* infections rests on isolation and identification of the suspected species from feces, urine, blood, or contaminated food.

In typhoid fever the organism can generally be isolated from the blood during the first week of illness. Later it may be isolated from the urine or stools. The suspected samples are first plated on the various media, as indicated under Isolation of the Enterobacteriaceae earlier in this chapter. The isolated species is then subjected to biochemical tests (see Table 23-3), followed by serological tests of the O, H, or Vi antigens.

The O (somatic) antigen has been assigned arabic numbers from 1 to 64. For convenience the salmonellae have been placed in serogroups A through Z, each group containing specific O antigens. For example, one serotype in group A might contain O antigens 1, 4, 12, and 27 while another serotype in group H might contain O antigens, 1, 6, 14, and 25. For identification of the most important salmonellae a commercial grouping serum is available. Nearly 95 percent of the serotypes isolated from humans and animals can be found in groups A through O. Some groups contain common antigens, and single-factor antiserum is available for more specific identification.

The *Vi* antigen is a special heat-labile K antigen that surrounds *S. typhi* and can prevent agglutination of O antigens. When negative reactions occur with O grouping sera, the Vi antiserum should be applied.

Confirmation of O or somatic composition rests on identification of the H or flagellar antigen. Most salmonellae have two different H antigens. The H antigens are designated by a lowercase letter: a, b, c, and so on. The two different H antigens occur in phases, which are genetically controlled and can be influenced by the age and composition of the culture medium. Speciation of salmonellae requires identification of both phases.

In gastroenteritis, specimens of feces, vomit, or foodstuffs are analyzed in the laboratory. The same procedures that are used for the enteric fever group can be applied. Phage typing is used epidemiologically to match patient isolate with contaminated food.

Blood samples are also used to isolate *Salmonella* species involved in septicemia.

TREATMENT

Salmonella infection responds to four drugs: chloramphenicol, ampicillin, amoxicillin, and trimethoprim-sulfamethoxazole. For gastroenteritis, antibiotics are contraindicated unless chronic bacteremia is also present. Replenishment of lost fluids and electrolytes is the most important aspect of gastroenteritis management, particularly for young children.

For enteric fevers and bacteremia, chloramphenicol is the best choice for antimicrobial therapy. Ampicillin is used if the species is resistant to chloramphenicol. Surgery may be required if there has been perforation of the ileum.

PREVENTION AND CONTROL

Salmonella infections can be controlled or at least reduced by (1) proper sanitation with special emphasis on sewage disposal and maintenance of unpolluted water supplies; (2) supervision of the preparation of products by the food industries; (3) proper refrigeration of foods, particularly poultry and related products; and (4) detection of asymptomatic carriers and their removal from occupations involved in the handling of food or care of the hospitalized until the carriers are adequately treated.

S. typhi vaccines are seldom administered in the United States; however, they are employed for military personnel and those traveling to areas where typhoid fever is endemic. The vaccine used is an acetone-killed suspension of *S. typhi* that has been shown to give a high degree of protection.

The vaccines used in most parts of the world where typhoid fever is endemic are administered parenterally. These vaccines frequently cause adverse reactions such as inflammation, fever, and headache. An oral vaccine was used in a controlled field trial in Alexandria, Egypt, from 1978–1981. The vaccine has proved safe and protective for a period of at least 3 years.

SHIGELLA SPECIES

Species of *Shigella* are causal agents of *bacillary dysentery*. With few exceptions the shigellae are harbored by humans and transferred by the fecal-oral route. Shigellae are also important historically because they were the first recognized microorganisms isolated from humans that carried multiple antibiotic resistance markers on plasmids.

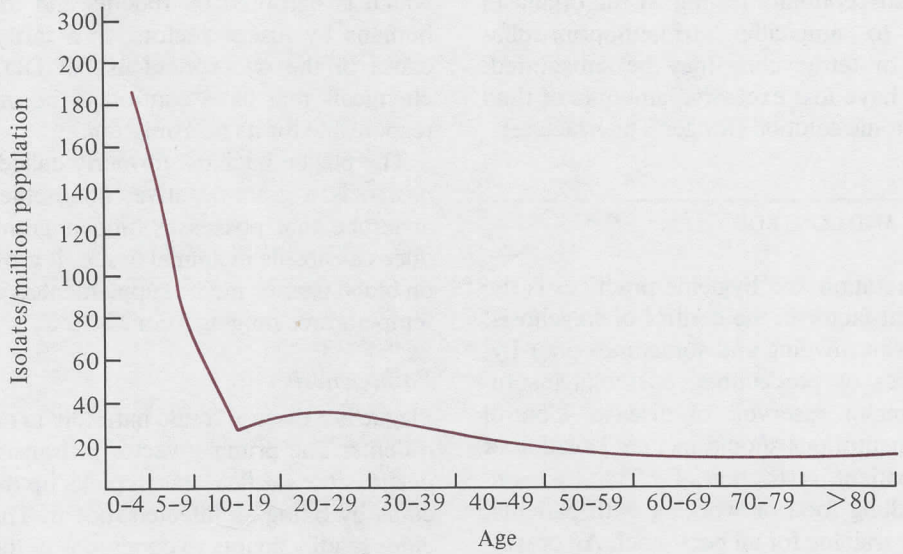
PATHOGENESIS

Like the salmonellae, the shigellae are transferred from person to person by contaminated food, water, or fomites. The incubation period for shigellosis is 36–72 hours. As few as 200 ingested *Shigella* organisms are enough to initiate infection. The shigellae invade the intestinal epithelium like the invasive *Salmonella* species. Shigellae, however, unlike the salmonellae, are found free in the cytoplasm of the epithelial cell. It appears that when engulfed by the cytoplasmic membrane of the epithelial cell the shigellae digest the membrane of the vacuole. Shigellae then spread laterally to invade adjacent epithelial cells. Epithelial invasion is not sufficient to cause disease; the organism must also replicate and be capable of persisting in the mucosa. A transient inflammation may result from penetration of the epithelium, but the mucosal ulceration associated with disease occurs only after microbial multiplication. Up to 10^{10} organisms can be found per gram of stool during disease. Abdominal cramps, fever, and watery diarrhea occur early in the disease. Dysentery occurs during the ulceration process with high concentrations of neutrophils in the stools.

The symptoms of shigellosis are mild or severe depending on the species causing infection. The severest forms of shigellosis are caused by *S. dysenteriae* type 1, also called the *Shiga bacillus*. In addition to its endotoxin, this species produces an enterotoxin that is associated with cytotoxic activities. The Shiga bacillus is capable of invading the bloodstream and causing fatal septicemia. The less virulent species such as *S. sonnei* and *S. flexneri* produce similar toxins but in much smaller amounts than *S. dysenteriae*. Recent studies with the cytotoxin using cultural HeLa cells (malignant cells originally obtained from a woman with cervical cancer) have revealed new aspects of *Shigella* pathogenesis. The toxin binds to a glycoprotein receptor on the epithelial cell surface and is translocated across the cytoplasmic membrane by an endocytic process. Inside the phagolysosome the toxin is believed to be processed into an active form. Active toxin inactivates the 60S ribosomal subunit and inhibits protein synthesis.

Most cases of shigellosis are self-limiting. However, young infants may die if there is severe loss of fluids.

Fig. 23-7. Rate of reported isolates of *Shigella* by age. (From Centers for Disease Control, Atlanta, GA.)



EPIDEMIOLOGY

The number of cases of shigellosis in 1982 was 13,523, which represents a steady decline from the high of 15,334 in 1978. *S. sonnei* accounted for 62.4 percent of the serotypes, followed by *S. flexneri* (32.5 percent), *S. boydii* (2.3 percent), and *S. dysenteriae* (1 percent). The highest rates of shigellosis are in the western states and among children from 1–5 years of age (Fig. 23-7).

Epidemics of shigellosis occur in crowded communities. Many cases are associated with day-care centers, prisons, and institutions for the mentally retarded. Military field groups and travelers to countries with unsanitary conditions are likely victims. (See boxed essay, p. 531.)

LABORATORY DIAGNOSIS

As a presumptive test for shigellosis some clinicians examine fecal specimens for the presence of sheets of neutrophils. Confirmatory tests are carried out by plating the specimens on various selective or differential media. Isolated species are then subjected to specific biochemical tests. Species that are nonmotile, do not ferment lactose, produce no gas from carbohydrate fermentation, and fail to produce hydrogen sulfide are possibly *Shigella*. Isolated colonies are then reacted with group-specific antisera for final confirmation.

Colicin typing of strains of *S. sonnei* is performed in some laboratories. The colicin determinants appear to be relatively stable in the various serotypes. Colicin typing is based on ability to produce colicins and not susceptibility to colicins.

TREATMENT

Although most cases of shigellosis are mild and do not require antibiotics, the latter can shorten the duration of diarrhea and eliminate the bacillus from the stool, preventing possible spread to others in

the community. Ampicillin is the drug of choice, but because of the widespread appearance of antibiotic resistance factors clinical isolates should be subjected to susceptibility testing. If the organism is resistant to ampicillin, trimethoprim-sulfamethoxazole or tetracycline may be substituted. Patients who have lost excessive amounts of fluid require an isotonic solution (Ringer's plus lactate).

PREVENTION AND CONTROL

Improving sanitation and hygienic practices is the most important factor in the control of shigellosis. Because of overcrowding and sometimes poor hygienic practices or procedures, custodial institutions are a major reservoir of disease. Control measures in institutions should include isolation of the infected patient, restriction of infected personnel from handling food or working with patients, and in-service training for all personnel. An oral attenuated *Shigella* vaccine has been very successful in reducing epidemics in custodial institutions. The vaccine consists of streptomycin-dependent *Shigella* strains that cannot multiply in the intestine but are capable of stimulating antibodies.

YERSINIA SPECIES

The genus *Yersinia* (formerly *Pasteurella*) has three important pathogenic species: *Y. pestis, Y. pseudotuberculosis*, and *Y. enterocolitica*. *Y. pestis* is the causative agent of *bubonic plague* (black death) in humans. *Y. pseudotuberculosis* causes epizootic disease in animals and is a minor human pathogen. *Y. enterocolitica* is now being recognized as an important human pathogen.

YERSINIA PESTIS

In the fourteenth century bubonic plague was responsible for the deaths of more than 25 percent of the population of Europe. The most recent pandemic occurred in Asia in 1904, where more than a million people succumbed. Today the disease, which is harbored by rodents and transmitted to humans by insect vectors, is a rarity, partly because of the widespread use of DDT and other chemicals that have controlled the insect vectors responsible for its transmission.

The plague bacillus, formerly called *Pasteurella pestis*, is a gram-negative, nonmotile, facultative anaerobe that possesses bipolar granules. It produces a capsule in animal tissue. It grows optimally on blood agar or media supplemented with blood at temperatures ranging from 27–30°C.

Pathogenesis

Plague is a disease found naturally in rats and other rodents. The primary vector in transmission of *Y. pestis* is the rat flea, which picks up the plague bacillus by biting an infected rodent. The plague bacillus readily adapts to conditions in the alimentary tract of the flea, where it multiplies and accumulates. The bacilli are then transmitted to humans through the bite of the infected flea. During the incubation period, which may last from 1–5 days, the patient is subject to vomiting and high fever. From the site of the fleabite the bacilli migrate to regional lymph nodes, such as axillary and inguinal nodes, causing their enlargement. The enlarged nodes are called *buboes*; hence the term *bubonic plague*. The bacilli proliferate in the mononuclear phagocytes and there develop antiphagocytic factors: a capsule called the *Fra factor* and two antigens called the *V* and *W* factors. The enlarged lymph nodes contain numerous bacilli and bacterial products, which have free access to lymphatic tissue. If the plague bacilli enter the circulation (septicemic plague), they can infect a number of different organs. On rare occasions the plague bacilli infect the lungs (pneumonic plague), producing massive hemorrhages. From the infected lungs the plague bacilli can enter the bloodstream. All forms of plague can be fatal, but the pneumonic type is invariably the most lethal if left untreated, on account of the toxemia caused by the lipopolysaccharide endotoxin of *Y. pestis*.

HOSPITAL-ASSOCIATED OUTBREAK OF *SHIGELLA DYSENTERIAE* TYPE 2—MARYLAND

An outbreak of severe dysentery caused by *Shigella dysenteriae* type 2 recently occurred at the U.S. Naval Hospital, Bethesda, Maryland. Epidemiologic investigation implicated the salad bar in the active-duty staff cafeteria as the source of infection.

In March 1983, 95 (6 percent) of 1490 active-duty hospital staff members and 12 other individuals (3 hospital inpatients, 4 visitors, and 5 food-service workers) became ill with acute dysentery. Onset of illness occurred over an 11-day period, and the epidemic curve was consistent with a common-source outbreak. Patients had chills, fever, abdominal cramps, and the abrupt onset of profuse watery or bloody diarrhea. Nausea, vomiting, myalgia, and dehydration were frequently noted. A case was defined as a patient with two or more of the following: (1) fever or chills, (2) nausea or vomiting, (3) watery or bloody diarrhea (more than two nonformed bowel movements per day), or (4) abdominal cramps. Twenty-four individuals required hospitalization for intravenous hydration. The duration of illness for most persons ranged from 3–8 days.

S. dysenteriae type 2 was cultured from stool specimens of 36 of the 80 affected individuals whose specimens were cultured. All symptomatic individuals were treated with a 5-day course of either ampicillin or trimethoprim-sulfamethoxazole, both of which were effective against the organism in vitro. One hundred three of 107 known symptomatic individuals and 102 controls matched by job category were interviewed, and food-specific histories were obtained. Eating food prepared in the staff cafeteria, where 900 to 1300 persons eat one or more meals daily, was significantly associated with illness. Analysis of food histories from patients and controls who ate at the cafeteria at any time between February 28 and March 3 showed that patients were significantly more likely than controls to have eaten raw vegetables from the salad bar. A single batch of salad vegetables prepared on February 28 was served at the salad bar through March 3. No single salad item or dressing was specifically implicated. No samples of salad from the days in question were available for culture, and no cultures taken from available food items were positive. No other outbreaks of food-related shigellosis were reported to state health authorities during the outbreak period.

Interviews and stool cultures were obtained from 63 food handlers. Five had illnesses meeting the case definition with onset concurrent with the other cases. Although no index case was identified, gastrointestinal illnesses appeared to be common causes of absenteeism among food handlers during the 3 weeks preceding the outbreak. No food handler had a positive stool culture; none reported recent emigration or foreign travel.

The scope of the outbreak was undoubtedly limited by the exclusion of civilians from eating in the cafeteria and because food for inpatients is

prepared in separate areas by different personnel. Although a few staff members continued to work while ill, most did not work after onset of symptoms. Only ambulatory inpatients or visitors who ate in the staff cafeteria became ill. Two persons with presumed secondary cases who denied eating in the cafeteria were identified, including a civilian nurse who reported taking a rectal culture 12 hours before onset of her own symptoms from a hospitalized patient with a case, and a student laboratory technician who had contact with other ill technicians.

Preliminary food histories led to closing the salad bar, the cold-sandwich line, and a self-service ice cream machine on March 7; these were reopened on March 14. Symptomatic health care workers rapidly improved with antibiotic therapy and were allowed to return to work 48 hours after symptoms had subsided. Symptomatic food handlers were required to have two negative rectal cultures, 24 hours apart, after completion of antibiotic therapy. The impact of this outbreak on the hospital operation was considerable.

S. dysenteriae type 2 is an uncommon cause of disease in the United States; in 1982, only 0.2% (20 of 8939) of the *Shigella* isolates of known serotype reported to the Centers for Disease Control were type 2.

Because the infectious dose of *Shigella* is relatively small, it is more likely to spread person to person than are other enteric pathogens such as *Salmonella* and *Vibrio cholerae* O1. Although 6 percent of the active-duty hospital staff members became ill, there was no evidence of secondary spread from staff to patients. The opportunity for spread from staff to patients was undoubtedly decreased since most ill staff members did not work after onset of symptoms.

From *MMWR* 32:250, 1983.

Actually five determinants of virulence have been identified in the *Yersinia*. It appears that all act in concert to aid the microorganism in pathogenesis and avoidance of host defenses:

1. Pur factor. The inability to synthesize adenine and guanine by *Yersinia* results in loss of virulence. Apparently Pur mutants are unable to transport purine ribotides or deoxyribotides and there are insufficient free purines or purine ribosides within the host cell to support growth.
2. Fra factor. *Y. pestis* produces a glycoprotein capsule called *fraction 1* (Fra^+) that confers resistance to phagocytosis. Fra^- mutants, however, show only reduced virulence, which indicates that other factors contribute to phagocyte resistance.
3. Pesticin. Most *Y. pestis* isolates produce a bacteriocin called pesticin (Pst). Pst^- cells exhibit reduced virulence when injected by peripheral routes into laboratory animals, but their virulence is the same as Pst^+ cells when injected intravenously. This result implies that the Pst determinant influences the ability of the pathogen to invade deep tissue of the host.
4. Pigment. *Y. pestis* absorbs chromophores such as hematin from the culture medium and produces pigmented (Pgm) colonies. A 10^7 to 10^8 dose of Pgm^- mutants is required to produce an LD_{50} in laboratory animals, as compared to a dose of less than 10 for the prototrophic strains.
5. Calcium. A requirement for calcium (Cal^+) in the growth medium has been associated with the production of the plague virulence antigens V

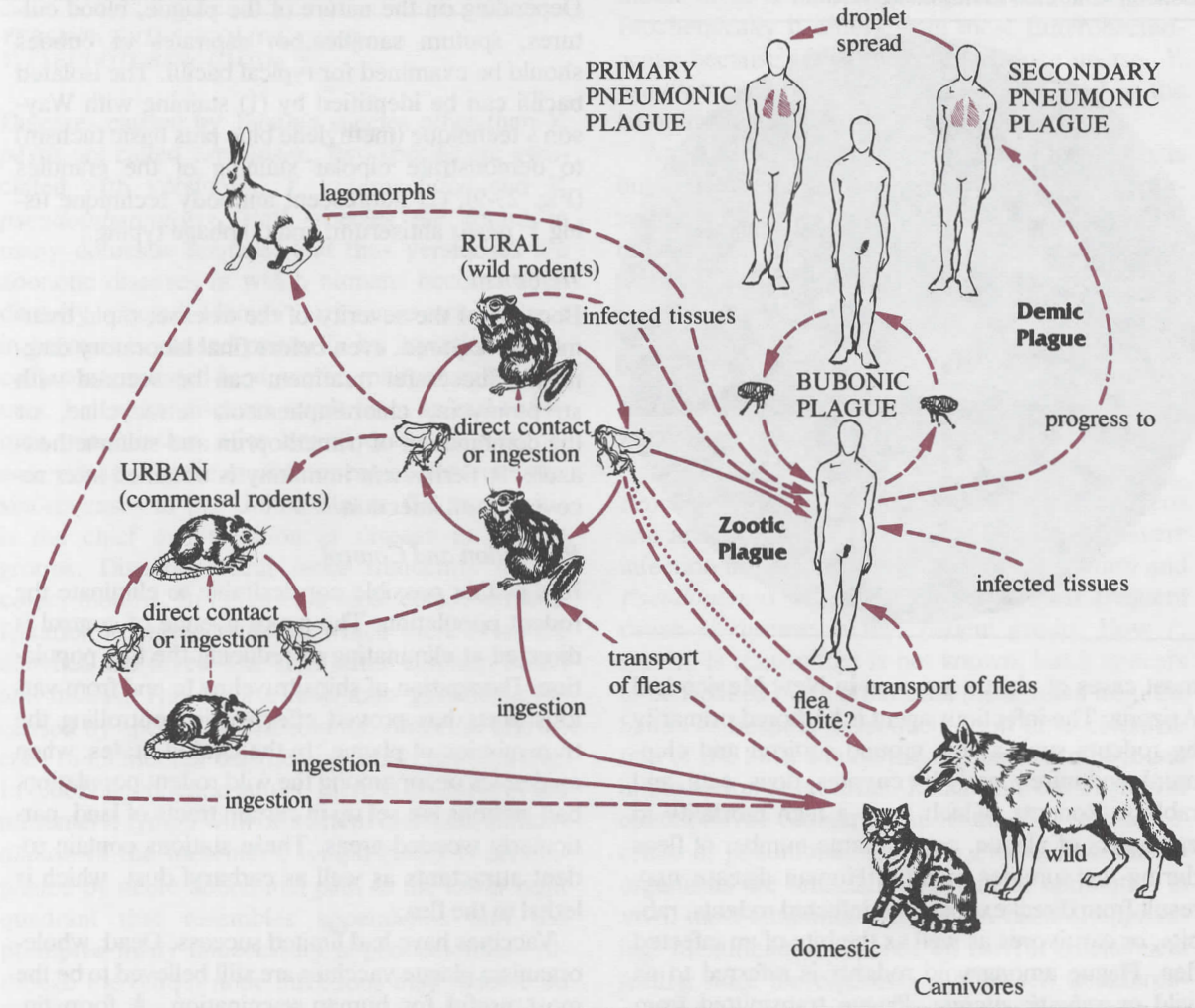


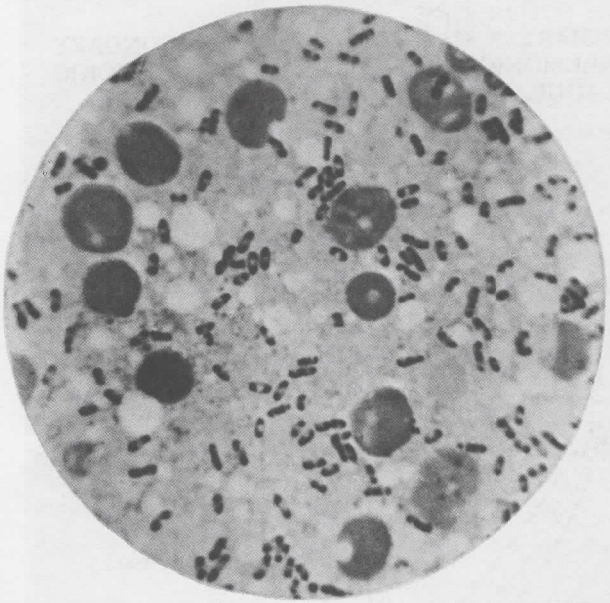
Fig. 23-8. Epidemiology of infection by *Yersinia pestis*. (*Solid lines* = usual pathway; *dashed lines* = occasional pathway; *dotted lines* = rare or theoretical pathway.) (From J. Poland. In P. Hoeprich [Ed.], *Infectious Diseases*. Hagerstown, MD: Harper & Row, 1977.)

and W. The gene associated with calcium dependence is located on a plasmid. Whether other virulence determinants are also located on the plasmid and not associated with calcium dependence is not known. The V and W antigens are believed to enable the pathogen to inhibit phagocytosis and to enhance multiplication in the phagocyte. The plasmids associated with calcium dependence and the V and W antigens are also found in *Y. enterocolitica* and *Y. pseudotuberculosis*.

Epidemiology

Plague, primarily a disease of rodents, is transmitted from animal to animal or animal to human by infected fleas (Fig. 23-8). In bubonic plague, rats are the primary reservoir of *Y. pestis*. Most cases are found in Southeast Asia. In the United States

Fig. 23-9. Smear prepared from lymph gland for demonstration of *Yersinia pestis*. Note bipolar staining. (From R. R. Gillies and T. C. Dodds, *Bacteriology Illustrated*. London: Churchill Livingstone, 1976.)



most cases of plague are seen in New Mexico and Arizona. The infectious agent is harbored primarily by rodents such as the ground squirrel and chipmunk and infrequently by coyotes, dogs, cats, and rabbits. Rodents, which have a high mortality in epizootics of plague, carry a large number of fleas during the summer months. Human disease may result from direct exposure to infected rodents, rabbits, or carnivores as well as the bite of an infected flea. Plague among wild rodents is referred to as wild or *sylvatic plague*. Plague transmitted from person to person is called *demic plague*. (See boxed essay, p. 536.)

Pneumonic plague, in which quantities of bacilli are found in the sputum, can be transmitted to others by respiratory droplets. Pneumonic plague is seen infrequently today but when it does occur it is usually an extension of septicemia and is seldom due to inhalation of the pathogen. Primary pneumonic plague is occasionally the result of contact with household pets who have plague pneumonia.

Thirty-nine cases of plague were reported in 1983 in the United States, with five fatalities.

Laboratory Diagnosis

Depending on the nature of the plague, blood cultures, sputum samples, or aspirates of buboes should be examined for typical bacilli. The isolated bacilli can be identified by (1) staining with Wayson's technique (methylene blue plus basic fuchsin) to demonstrate bipolar staining of the granules (Fig. 23-9), (2) fluorescent antibody technique using *Y. pestis* antiserum, and (3) phage typing.

Treatment

Because of the severity of the disease, rapid treatment is required, even before final laboratory diagnosis. Successful treatment can be secured with streptomycin, chloramphenicol, tetracycline, or the combination of trimethoprim and sulfamethoxazole. A permanent immunity is obtained after recovery from infection.

Prevention and Control

It is neither possible nor desirable to eliminate the rodent population. The major avenue of control is directed at eliminating or reducing the flea population. Fumigation of ships traveling to and from various ports has proved effective in controlling the transmission of plague. In the United States, when epidemics occur among the wild rodent population, bait stations are set up in certain tracts of land, particularly wooded areas. These stations contain rodent attractants as well as carbaryl dust, which is lethal to the flea.

Vaccines have had limited success. Dead, whole-organism plague vaccines are still believed to be the most useful for human vaccination. A formalin-killed vaccine is currently being used for people working in areas where plague is endemic. Vaccination is recommended for laboratory personnel who are working with *Y. pestis* organisms resistant to antimicrobials, persons engaged in aerosol experiments with *Y. pestis*, and persons working in areas with enzootic plague. Vaccination should also be considered for laboratory personnel working with plague-infected rodents and those whose occupation or residence brings them into continual

contact with wild rodents or rabbits in enzootic areas.

YERSINIA ENTEROCOLITICA AND *Y. PSEUDOTUBERCULOSIS*

Diseases caused by *Yersinia* species other than *Y. pestis* are called *yersinoses*. Two species are associated with yersinosis, *Y. enterocolitica* and *Y. pseudotuberculosis*. Both species are found in many domestic animals, and thus yersinoses are zoonotic diseases in which humans become accidentally infected. Human carriers are rare. Disease in humans is caused primarily by ingestion of fecally contaminated food, water, milk, or milk products, but some diseases result from animal-to-human transmission. (See boxed essay, p. 539.) *Y. enterocolitica* is the most frequent isolate from yersinosis cases in the United States. Gastroenteritis is the chief manifestation of disease in all age groups. Diseases occur more frequently in the colder months. In infants the most common manifestation is enterocolitis, in which there is severe diarrhea and fever. The stool often contains blood and mucus. The disease resembles gastroenteritis caused by species of *Salmonella, Shigella,* and *E. coli*. In infants the diarrhea is usually self-limiting. In older children and adults, there is invasion of the mesenteric lymph with or without diarrhea. Inflammation of the mesenteric lymph nodes is accompanied by acute abdominal pain in the lower right quadrant that resembles appendicitis and has prompted many unnecessary appendectomies. Although the lymph node infections may resolve on their own, in some individuals, particularly Europeans, either the infection develops into arthritis involving the ankles, fingers, and knees and lasting up to 4 months, or it may lead to erythema nodosum, with subcutaneous nodules appearing, usually on the legs. The latter terminates without treatment but leaves discolored areas on the skin. In the compromised host, *Yersinia* infections can be devastating; the fatality rate in septicemia, for example, is higher than 50 percent.

Y. enterocolitica is usually overlooked in laboratory diagnosis unless it is specifically sought. It is difficult to isolate from stool specimens because of the presence of other enteric species. It is actively motile at 20°C but sluggish or nonmotile at 37°C. Biochemically it differs from most Enterobacteriaceae because of its ability to produce urease. *Y. pseudotuberculosis* is more easily isolated in the laboratory and will grow well on blood agar.

Antibiotics are not required for gastroenteritis but rather only replacement of lost fluids. Chloramphenicol, gentamicin, tobramycin, and the combination trimethoprim-sulfamethoxazole are all effective in the treatment of systemic disease.

PROVIDENCIA AND *ARIZONA* SPECIES

Providencia species are opportunistic pathogens and are recognized as important causes of severe infection in burn patients. *Providencia stuartii* and *Pseudomonas aeruginosa* are the most frequent cause of disease in that patient group. How *P. stuartii* is transmitted is not known, but it appears to be isolated more often from the air than from the hands of hospital personnel. Soon after colonization of the burn wound the organism can be found in the sputum and urine. Fatalities from *P. stuartii* infections are ordinarily the result of severe bacteremia or pneumonia. Because *Providencia* microorganisms are unusually resistant to antibiotics in vivo, most antibiotic therapy has been disappointing. Identification depends on careful biochemical testing since the organism is often left undifferentiated from other members of the Enterobacteriaceae.

Arizona species, which are harbored by many animal species, are pathogenic for snakes, turkeys, and chickens and are opportunistic pathogens for humans. Snakes and rats are a potential reservoir of infection for humans. Diseases caused by *Arizona* species resemble those caused by salmonellae, that is, gastroenteritis, enteric fever, and bacteremia. Food handlers who carry *Arizona* species in their feces and ingestion of contaminated dairy

PLAGUE—SOUTH CAROLINA

On August 5, 1983, plague was diagnosed in a 13-year-old girl in South Carolina. She became ill while en route to Maryland from her previous residence in Santa Fe, New Mexico, and subsequently died. The area in which she had lived had been recognized as a locality where sylvatic plague was enzootic.

On July 25, the girl, a horsewoman who spent considerable time outdoors, handled and then released a wild chipmunk. On July 27, she flew to Atlanta, Georgia, and spent the night with friends; the following day she was driven to Seneca, South Carolina. That evening, she complained of a sore throat and tenderness in her right groin and reportedly had a temperature of 40.0°C (104°F). On July 29, she saw a physician, who noted an oral temperature of 38.3°C (101°F), pharyngeal erythema, tender cervical lymph nodes and a 1-×-2-cm tender right inguinal lymph node. Laboratory tests, including complete blood count, urinalysis, and throat culture, and tests for mononucleosis were done, and oral penicillin was prescribed. Three days later she was seen again, still febrile and with expanding right inguinal nodes. Her white blood cell count was 20,500, and a chest x-ray was normal. Because of her history of residence in a plague-enzootic state, a diagnosis of plague was considered. She was hospitalized and given parenteral therapy, including streptomycin. By the following morning, she was tachypneic, with productive bloody sputum, and appeared moribund. She was transferred to a large, regional medical center where, despite intensive supportive care and therapy with intravenous chloramphenicol, she developed overwhelming sepsis and died on August 2. A chest radiograph taken before death revealed extensive pulmonary infiltrates.

Antemortem aspiration of the right inguinal lymph node demonstrated gram-negative bipolar-staining bacilli on Giemsa stain. Both this aspirate and multiple cultures of blood yielded *Yersinia pestis*. In addition, fluorescent antibody (FA) stains for *Y. pestis* were positive for specimens consisting of blood smears, culture material, and pulmonary secretions.

Based on the clinical picture and the positive FA results from sputum, it appears that pneumonic plague and the potential for human-to-human transmission existed terminally. Local health care providers had placed her in complete isolation before this development. Hospital staff directly in contact with her at this point were given prophylactic tetracycline and followed up for evidence of illness. No secondary cases appeared during the expected incubation period.

Primary pneumonic plague in the United States has been described as rare, with only three cases between 1926 and 1977—all in laboratory workers. Recent investigations suggest that plague pneumonia (i.e., secondary to bubonic plague) is more common. Thus far in 1983, 24 cases of human plague have originated in New Mexico, and three (13 percent) of them have had pneumonic involvement. No transmission to contacts of patients with pneumonic plague has been documented in the United States since 1925.

From *MMWR* 32:417, 1983.

products have been implicated in outbreaks. Carriers of *Arizona* species usually harbor the organism in the gallbladder. *Arizona* species resemble *Salmonella* species serologically and biochemically, but *Arizona* differs biochemically in that it shows delayed lactose fermentation and delayed gelatin hydrolysis and it does not ferment dulcitol. As with *Salmonella* species, chloramphenicol and ampicillin are the drugs of choice for treatment of disease.

ENTEROBACTER SPECIES

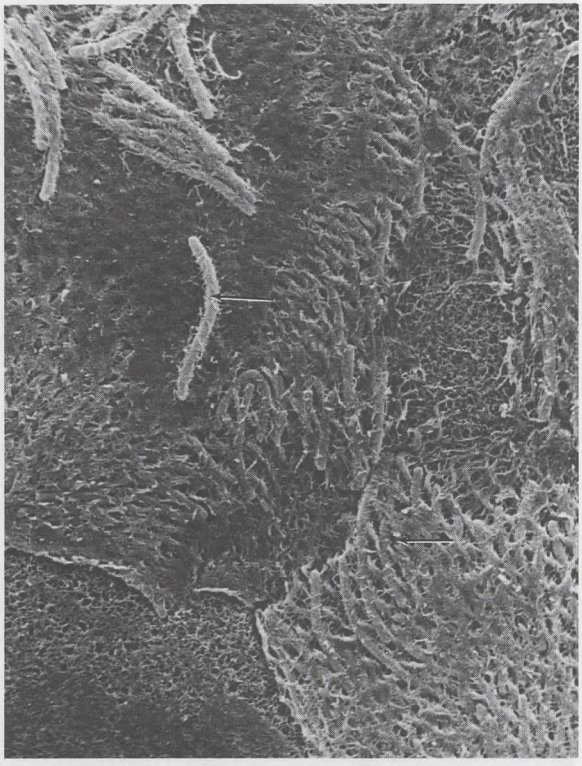
Enterobacter species are found in soil, water, dairy products, and the intestinal tracts of humans and animals. *E. sakazakii*, the most frequently isolated species from human disease, is associated primarily with the urinary tract. Most infections occur in hospitalized patients who are seriously ill. Several hospital-associated outbreaks have also occurred in burn units where spread is associated with patient handling by hospital personnel. Most strains appear to produce beta-lactamases; therefore treatment usually involves administration of aminoglycosides, chloramphenicol, and tetracyclines but not penicillins.

PROTEUS SPECIES

Species of *Proteus* are opportunistic pathogens found in soil, water, and the human intestinal tract. The most important species pathogenic to humans are *P. mirabilis, P. vulgaris*, and *P. rettgeri*. *P. mirabilis* is the most frequently isolated human pathogen. Most *Proteus* diseases are acquired in the hospital. The major type of *Proteus* infection in the community involves the urinary tract, whereas in the hospital infections may involve the blood, soft tissue, lower respiratory tract, and urinary tract. *P. rettgeri* has been associated with severe urinary tract infection in hospitalized patients. Transmission of the infectious agent appears to be due to hand transfer by hospital staff during the manipulation of catheters. Most *Proteus* species are susceptible to ampicillin, cephalothin, and the aminoglycosides. Like other enteric species, they carry R factors, and susceptibility tests should be done on clinical isolates.

Species of *Proteus* are among the most actively motile microorganisms and have a characteristic tendency to swarm on most laboratory media (Fig. 23-10). Isolation requires initial cultivation on EMB or MacConkey agar followed by transfer to TSI agar slants. This is followed by inoculation on urea

Fig. 23-10. Scanning electron micrograph of *Proteus mirabilis* on an agar surface. Many of the bacterial cells are matted together (*arrow, lower right*), but others are more isolated (*arrow, center*) and can be seen to be peritrichously flagellated. (From S. Stahl, K. R. Stewart, and F. Williams, *J. Bacteriol.* 154:930, 1983.)



agar. One of the distinguishing characteristics of *Proteus* species is their ability to produce the enzyme urease, which converts urea to CO_2 and ammonia.

KLEBSIELLA SPECIES

Klebsiella are opportunistic pathogens for humans. Four species of *Klebsiella* are associated with human diseases: *K. pneumoniae, K. ozaenae, K. oxytoca*, and *K. rhinoscleromatis*. *K. ozaenae* and *K. rhinoscleromatis* are indigenous colonizers of the nasopharynx of humans. *K. pneumoniae*, however, can be found in the throat and intestine of only 10 percent of healthy adults. The percentage increases markedly in patients who are in the hospital for extended periods. Ninety-five percent of the clinical isolates of *Klebsiella* are *K. pneumoniae*; the remainder, *K. oxytoca*. In the hospital *K. pneumoniae* is one of the major causes of septicemia in pediatric wards. It is also responsible for bacterial pneumonia and upper respiratory tract infections in patients already compromised by upper respiratory tract diseases. *K. pneumoniae* is a prime suspect as the cause of pneumonia in alcoholics. It is particularly destructive to lung tissue, resulting in the formation of abscesses that may require surgical removal.

K. pneumoniae is distinguished from other members of the enteric bacilli because it is nonmotile and produces a large mucoid colony on most laboratory media. A lactose fermenter, it can be cultivated on enteric isolation media such as EMB or MacConkey agar. More than 70 capsular types of *K. pneumoniae* have been identified with K type–specific antisera. Greater accuracy in identification of specific types is obtained by means of the quellung reaction. *K. pneumoniae* is sensitive to a number of antibiotics: kanamycin, gentamicin, amikacin, chloramphenicol, and streptomycin. Hospital strains have been isolated that show resistance to several drugs. The resistance pattern is usually determined by the type of antibiotic regimen used by the hospital over long periods of time. Most resistance patterns are due to the carriage of R factors.

K. ozaenae is now being recognized as an important pathogen that may cause infections of the middle ear, urinary tract, and soft tissue. It is also believed to be a possible cause of *ozena*, a chronic rhinitis characterized by atrophic changes in nasal mucosa resulting in the production of a foul-smelling mucopurulent discharge.

SERRATIA MARCESCENS

S. marcescens is an opportunistic pathogen that is a common saprophytic microorganism found in soil and water but rarely in the intestinal tract of humans or animals. Some strains produce a water-insoluble red pigment. (Strains isolated in the hospital are rarely pigmented.) Since the advent of antibiotics *S. marcescens* has become increasingly important in nosocomial infections. Patients with indwelling catheters and those undergoing antibiotic chemotherapy are high risks for *Serratia* infection. *S. marcescens*, a hardy microorganism, has been found in the handwashings of hospital personnel, in saline irrigant solutions, and in other so-called sterile solutions. Hospital infections probably occur mainly by transfer from patient to patient via hospital personnel. Most strains are resistant to many of the common antibiotics used in chemotherapy. After its isolation the organism should be tested for its sensitivity to the various antibiotics. Identification of nonpigmented strains requires biochemical testing. Gentamicin, carbenicillin, and kanamycin are useful in the treatment of most *Serratia* infections.

SUMMARY

The Enterobacteriaceae are gram-negative facultative anaerobes that possess pili on their surface and

OUTBREAK OF *YERSINIA ENTEROCOLITICA*—WASHINGTON STATE

In December 1981 and January 1982, an outbreak predominantly of gastroenteritis caused by *Yersinia enterocolitica* occurred among 87 persons in Washington State. The illness was associated with the ingestion of a locally produced brand of tofu, an oriental soybean curd, packed in untreated spring water. *Y. enterocolitica* was isolated from the tofu, the processing plant's water supply, and several sites within the plant.

In mid-January 1982, the Seattle–King County Health Department received reports from two hospital laboratories of 12 positive stool cultures of *Y. enterocolitica* associated with gastrointestinal illness; during the previous year, 10 *Y. enterocolitica* isolates were reported in the entire county. Increased surveillance by Seattle–King County and the Washington State Department of Social and Health Services over several months identified additional cases, for a total of 87.

Of the 87 cases, 56 were culture positive; 38 patients had enteritis, 6 had only extraintestinal infections, 4 had both extraintestinal infections and enteritis, and 8 were asymptomatic carriers. The 10 cases of extraintestinal infection included patients with wound ulcers (2), inguinal lymphadenopathy (2, 1 with a perineal ulcer), pneumonia (2), labial infection (1), arthritis (1), septicemia (1), and pharyngitis (1). Symptoms lasted from 1 day to 4 weeks (mean 10 days). Two patients, however, were ill for over 2 months. Seventeen patients were hospitalized for from 2 to 11 days (average 9.7 days); two of those hospitalized had appendectomies and one, a partial colectomy. One patient was also culture positive for *Salmonella typhimurium* and one for rotavirus, as well as for *Y. enterocolitica*.

A neighborhood case-control study of 11 ill persons and 11 controls revealed an association between *Y. enterocolitica* infection and tofu consumption. Further investigation revealed that 70 (80.5%) of the 87 persons interviewed had consumed the same brand of tofu within the 2 weeks before onset of symptoms. For 5 culture positive persons who had consumed only one meal of tofu, the incubation period averaged 6.6 days (range 4–11 days).

The tofu plant in King County is located on a rural island in Puget Sound. The plant water supply, which originates from a spring approximately 0.5 miles from the plant, is shared by four residences and an apple cider plant. No illness was reported among consumers of the cider. Inspection of the tofu plant on January 20, 1982, disclosed unsanitary conditions, including poor personal hygiene, use of an outdoor privy, and unsanitary equipment. Samples of tofu and the plant water supply were positive for *Y. enterocolitica*, as were stool specimens collected from 2 of 12 employees; both employees were asymptomatic.

A voluntary recall of the product was instituted from January 21 to January 25. Further sampling and laboratory analysis of the tofu demonstrated high fecal coliform counts. Production was resumed after a water purification system was installed.

From *MMWR* 31:562, 1982.

are able to ferment glucose. They are capable of producing bacteriocins and are classified according to their cell envelope–associated antigens: O, K and H antigens. They readily conjugate with other members of their group, and thus antibiotic-associated plasmids are readily transferred among the various species. Several species are true pathogens (*E. coli, Salmonella, Shigella*, and *Yersinia*), while others are opportunistic (*E. coli, Klebsiella, Serratia, Enterobacter*, and others).

The Enterobacteriaceae are easily isolated from clinical specimens. A variety of selective, differential, and enrichment media are available for their cultivation. Many biochemical tests as well as serological tests can be used for identification of individual species. Triple sugar iron agar represents one of the most important laboratory biochemical media for separation and presumptive identification of enteric bacilli.

Escherichia coli is an indigenous member of the intestinal tract, but some strains possess pathogenic potential for humans. Extraintestinal disease is frequently associated with compromised patients, and *E. coli* is one of the leading causes of disease in hospitals. Type 1 pili are important for adherence of these *E. coli* strains to epithelial cells. Intestinal disease is produced by three groups of *E. coli* strains referred to as enterotoxigenic, enteropathogenic, and enteroinvasive. Enterotoxigenic strains produce an exotoxin that stimulates adenyl cyclase in the intestinal epithelium and causes diarrhea. Pathogenic mechanisms for enteropathogenic and enteroinvasive strains are poorly understood. Treatment of intestinal disease involves the replenishment of fluids and electrolytes, while extraintestinal disease is treated with chloramphenicol and tetracyclines.

Salmonella species are a major cause of gastrointestinal disease and enteric fevers. They result from the ingestion of contaminated food or water. Diarrhea is associated with these gastrointestinal diseases and is presumably caused by stimulation of adenyl cyclase. Enteric fevers such as typhoid and paratyphoid fevers are caused by species that invade the intestinal epithelium and multiply in the macrophage and then invade the bloodstream. Some salmonellae can invade the gallbladder where they constitute a reservoir for shedding. The causes of enteric fevers are *S. typhi* and *S. paratyphi A* and *B*. Bacteremia is usually caused by *S. typhimurium* and *S. choleraesuis*. Salmonellae are identified by serological techniques. Treatment of gastrointestinal disease involves replenishment of fluids and electrolytes, while enteric fevers and bacteremia can be resolved by administration of chloramphenicol, ampicillin, amoxicillin, and trimethoprim-sulfamethoxazole.

Shigella species are a cause of bacillary dysentery and are transferred from person to person by contaminated food, water, or fomites. The most virulent species is *S. dysenteriae*, which is believed to produce an enterotoxin. Shigellosis is frequently encountered in crowded institutions in which the very young or very old are housed. Disease is usually characterized by diarrhea and abdominal cramps unless the bloodstream is invaded. Antibiotics are usually not recommended, but they can reduce the potential for spread by eliminating the bacilli.

The genus *Yersinia* contains several species that are important pathogens. *Y. pestis* is the cause of bubonic plague, a disease that is found naturally in rats and can be transmitted to humans by the bite of rat fleas. The plague bacillus infects lymph nodes near the site of infection to produce buboes, and then the organism enters the bloodstream to infect other organs. Pneumonic plague is the most lethal form of the disease. *Y. pestis* possesses several determinants that may aid in pathogenesis, but their actual contribution to virulence is still speculative. Diseases caused by *Yersinia* species other than *Y. pestis* are called yersinoses. These species include *Y. pseudotuberculosis* and *Y. enterocolitica*. These organisms are found in animals and are transmitted to humans in whom gastroenteritis is the principal symptom of disease. Streptomycin, chloramphenicol, and tetracycline are important antimicrobials for treatment of disease caused by *Yersinia*.

Other Enterobacteriaceae that are opportunistic pathogens include species of *Providencia, Arizona, Enterobacter, Proteus, Klebsiella*, and *Serratia*.

QUESTIONS FOR STUDY

1. List four characteristics that are common to the Enterobacteriaceae.
2. Which biochemical tests are useful for separating pathogens such as *Salmonella* and *Shigella* from the opportunistic pathogens such as *E. coli*?
3. What are the virulence determinants for enterotoxigenic *E. coli*?
4. How may phase variation in *E. coli* be related to virulence?
5. How does the treatment of gastrointestinal disease differ from that for extraintestinal disease caused by enteric bacilli?
6. Which *Salmonella* species are primary causes of gastroenteritis? Enteric fever?
7. Describe the mechanism used by *Salmonella* to penetrate the intestinal epithelium and subsequently enter the bloodstream.
8. Under what conditions might antibiotics be recommended for the treatment of shigellosis?
9. What factors influence the development of the carrier state after *Salmonella* infections?
10. What are the most common sources of nontyphoidal *Salmonella* infection in the United States?
11. What is the cause of bacillary dysentery? How do humans contract the disease?
12. What are the virulence determinants for *Yersinia pestis*?
13. Why would the administration of antibiotics late in *Y. pestis* infection prove ineffectual?
14. What kind of disease is associated with infection by *Y. enterocolitica* and *Y. pseudotuberculosis*?
15. List the principal genera of Enterobacteriaceae that are considered opportunistic pathogens.

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24. *PSEUDOMONAS* AND RELATED ORGANISMS

OBJECTIVES

To describe those virulence factors associated with *Pseudomonas* infections

To explain the techniques used to identify strains of *Pseudomonas aeruginosa*

To list the drugs most effective in treatment of *P. aeruginosa* infections and to explain why the organism is so resistant to most antimicrobials

To list some *Pseudomonas* species other than *P. aeruginosa* that are associated with human infection

Pseudomonas aeruginosa
General Characteristics
Pathogenesis
Proteases
Exopolysaccharide
Exotoxin A
Phospholipase
Laboratory Diagnosis
Treatment
Other *Pseudomonas* Species and Related Bacilli

The genus *Pseudomonas* belongs to a group of bacteria that are characterized as gram-negative, nonfermentative, aerobic bacilli. Pseudomonads are opportunistic pathogens and like other members of the group—*Alcaligenes, Achromobacter, Acinetobacter, Moraxella, Kingella, Flavobacterium, Eikenella*, and *Agrobacterium*—are associated with disease in the hospital setting. The most important species in terms of causing disease is *Pseudomonas aeruginosa*.

PSEUDOMONAS AERUGINOSA

GENERAL CHARACTERISTICS

P. aeruginosa rods are from 2 to 4 μm in length, and most are motile. *Pseudomonas* produces a slime layer at all times, but in certain instances an extracellular polysaccharide is produced by mucoid strains, for example, those strains found in the lungs of cystic fibrosis patients. Pseudomonads are known for their ability to catabolize a wide variety of carbon sources. They are found in soil and water and are able to colonize a number of habitats. The success of *P. aeruginosa* in colonizing and multiplying in a wide variety of environments is due to its biphasic growth patterns. Sometimes the organism exists as a highly motile cell capable of swimming from site to site; at other times it produces a slimy layer that enables it to grow in the form of an adherent microcolony. Another distinguishing characteristic of most *P. aeruginosa* mucoid strains is their ability to produce water soluble fluorescent pigments called *pyoverdins*. Only *P. aeruginosa* produces the nonfluorescent water soluble pigment called *pyocyanin*. Cultures of the organism, or wounds infected by it, give off a distinctive grapelike odor due to their pigments. The compound producing this odor is 2-aminoacetophenone.

PATHOGENESIS

P. aeruginosa is a ubiquitous organism, and most persons have antibodies to the species. Most *Pseudomonas* diseases are associated with those who are severely compromised. For example, *P. aeruginosa* has become the predominant organism isolated from patients with cystic fibrosis and severe lung diseases. The organism is also one of the leading causes of gram-negative infections of the urinary tract, surgical wounds, and burn wounds (Fig. 24-1). It is second only to *E. coli* as the major cause of hospital-associated disease. *P. aeruginosa* is well suited to its environment because it possesses components and produces products that are believed to be associated with virulence. We will discuss some of the more important virulence determinants. Some of the others are outlined in Table 24-1.

Proteases

The ability of *P. aeruginosa* to colonize the respiratory tract is correlated with its ability to adhere to respiratory epithelium. In vitro experiments have demonstrated that treatment of buccal (cheek) epithelial cells with a proteolytic enzyme results in increased adherence of *P. aeruginosa* cells. Experiments in vivo have demonstrated that in seriously ill patients there is a higher level of salivary protease than in healthy persons. This increased pro-

Fig. 24-1. Generalized invasive *Pseudomonas* burn wound sepsis in a child. (From B. A. Pruitt, et al., *Rev. Infect. Dis.* 5 [Suppl. 5]:889, 1983.)

tease level in ill patients is believed to be responsible for the loss of a cell surface component and the increased adherence of gram-negative bacilli such as *P. aeruginosa*. Treating burned mice with a serum protease inhibitor protects these animals from infection with *P. aeruginosa*.

Proteases can also dissolve gelatin, elastin, and collagen and when injected into the skin of animals can induce lesions and necrosis. Thus a variety of proteases may be involved in pathogenicity.

Exopolysaccharide

Mucoid strains of *P. aeruginosa* isolated from the respiratory tract of patients with cystic fibrosis produce an exopolysaccharide. The exopolysaccharide is an alginate composed of mannuronic-glucuronic acids. This polysaccharide is different in chemical composition from the polysaccharide slime produced by nonmucoid cells. The exopolysaccharide alginate allows the organism to form microcolonies. When artificially produced microcolonies are introduced into the lungs of normal rats, disease can be induced. The microcolony withstands clearance mechanisms in the host's respiratory tract and also inhibits phagocytosis. Host cells are killed by the microcolony, and the pathogens multiply and spread to form other microcolonies.

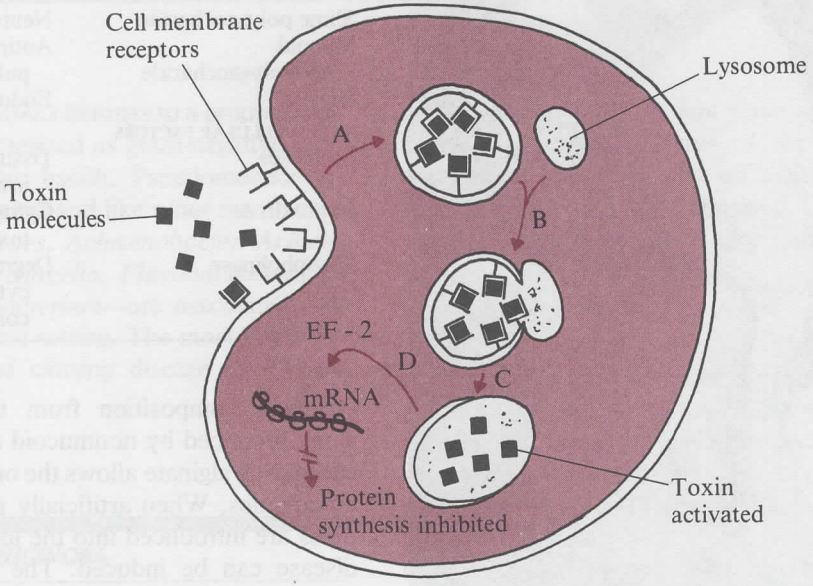
Table 24-1. Possible Virulence Determinants of *Pseudomonas Aeruginosa*

Determinant	Biological activity
CELLULAR FACTORS	
Pili	Adherence to epithelial cells
Slime polysaccharide	Neutrophil toxicity
Mucoid exopolysaccharide	Antiphagocytic, decreased pulmonary clearance
Lipid A	Endotoxic effects
EXTRACELLULAR FACTORS	
Proteases	Tissue adherence, tissue invasion and damage
Exotoxin A	Cytotoxicity, macrophage toxicity
Phospholipase	Degradation of cytoplasmic membrane components

Exotoxin A

Exotoxin A is an extracellular enzyme produced by most clinical strains of *P. aeruginosa*. The exotoxin is a protein composed of two polypeptide chains, A and B. Fragment B is the receptor-binding portion of the exotoxin A molecule and is believed to initiate the transport of fragment A, the enzymatic fragment. The intact polypeptide is probably transported across the cytoplasmic membrane by an endocytic mechanism (Fig. 24-2). Once inside the cytoplasm fragment A is separated from fragment B and becomes activated. Exotoxin A, like diphtheria toxin, is a proenzyme consisting of an enzymatically active NH_2 terminal portion (fragment A) and the COOH terminal binding portion (fragment B). Exotoxin A enzymatically catalyzes the

Fig. 24-2. Mechanism of exotoxin A transport and activation in the cell. A. Toxin binds to cell receptors and is transported into the cytoplasm within a vesicle. B. Lysosome fuses with the toxin-containing vesicle, and lysosomal activity activates the toxin. C. Activated toxin is released into the cytoplasm where it inhibits cellular protein synthesis by interfering with elongation factor (*EF2*).



transfer of the adenosine diphosphate ribosyl (ADPR) moiety from nicotinamide-adenine dinucleotide (NAD) to a covalent linkage with elongation factor 2 (EF2), which is involved in protein synthesis:

$$NAD + EF2 \xrightarrow{\text{exotoxin A}} ADPR\text{-}EF2 + \text{nicotinamide} + H^+$$

Thus ribosomal translocation along the messenger RNA (mRNA) is stopped, and protein synthesis inhibited.

After injection of exotoxin A into animals there is necrosis of liver cells followed by a decrease in cardiac output and systemic arterial blood pressure. Death results from respiratory failure. The exotoxin may also be immunogenic and affect human macrophages. The toxin is one-tenth as toxic as diphtheria toxin. Since many other products are released by *P. aeruginosa* it is difficult to assess the complete role of exotoxin in the disease process.

Phospholipase

Phospholipase is an extracellular product that liberates phosphorylcholine from lecithin, the component of eukaryotic membranes. Phospholipase is capable of causing pulmonary necrosis.

LABORATORY DIAGNOSIS

Clinical specimens from blood, burns, and other

sources can be cultured on ordinary media. Because *Pseudomonas* species are nonfermenters, their presence can be presumptively confirmed on triple sugar iron (TSI) agar by their inability to produce an acid butt or slant. However, many other tests are required for identification. The greenish blue colony color resulting from the production of pyocyanin and pyoverdins and the grape-like odor of cultures also aids in their identification. All strains are oxidase positive.

Phage typing, serotyping, and pyocin typing are three methods of identifying and establishing relationships among strains of *P. aeruginosa*. Phage typing is not always effective because many strains are resistant to lytic phage. Serotyping is of limited value because most strains fall into relatively few serotypes. *Pyocin* (a bacteriocin) typing is effective in determining the source of hospital-acquired infections. In this procedure the liquid pyocin in the broth culture is added to lawns of indicator strains, and growth inhibition is measured (see Chapter 13).

TREATMENT

P. aeruginosa is resistant to most antibiotics. The various mechanisms of antimicrobial resistance have been found to be combined in *P. aeruginosa*. This organism can produce beta-lactamase (plasmid-associated), has cell wall channels (porins) that are too small to allow transport of many antimicrobials, and contains altered penicillin-binding proteins. In vitro antibiotic sensitivity tests should be done before antibiotic therapy is begun. Most strains are susceptible to polymyxin B, colistin, and gentamicin, but these are very toxic and must be used sparingly. Severe infections are usually treated with carbenicillin, carbenicillin plus gentamicin, gentamicin, or tobramycin plus ticarcillin.

For patients with severe burns a polyvalent vaccine consisting of seven serotypes has been used with some success to prevent *Pseudomonas* infections.

OTHER *PSEUDOMONAS* SPECIES AND RELATED BACILLI

A number of other *Pseudomonas* species as well as related bacilli occasionally cause disease in humans. These species are described in Table 24-2.

SUMMARY

Pseudomonas is the most important member of a group of bacteria that are gram-negative, nonfermentative, aerobic bacilli. The species *P. aeruginosa* is one of the most important pathogens among compromised patients, such as burn patients, in the hospital environment. The organism is resistant to many antimicrobials and is capable of surviving in a variety of environments. *P. aeruginosa* produces diffusible blue-green pigments that give off a characteristic grape-like odor.

Cell- and host-associated factors have been discovered to be potential virulence determinants for *P. aeruginosa*. They include (1) the presence in ill patients of proteases that promote adherence of the microorganism to the epithelial cell surface; (2) exopolysaccharide formation by mucoid cells that allows the organism to produce microcolonies in host tissue; (3) production of exotoxin A, an extracellular enzyme that inhibits translocation in eukaryotic protein synthesis; and (4) extracellular phospholipase formation that promotes the degradation of lecithins in the cytoplasmic membrane.

P. aeruginosa can be presumptively identified on triple sugar iron agar, but a host of other biochemical tests are also required. Pyocin typing is an important technique for epidemiologic studies involving the microorganism.

Treatment of disease caused by *Pseudomonas* species must be preceded by antibiotic susceptibility tests. The organism possesses several mechanisms for resisting the activities of most antimicrobials. Most severe infections are treated with carbenicillin, gentamicin, tobramycin, and ticarcil-

Table 24-2. Characteristics of *Pseudomonas* Species Other than *P. aeruginosa* and Related Bacilli

Species	Disease association	Other characteristics
Pseudomonas fluorescens	Respiratory and urinary tract, wounds, bacteremia	Produces pyoverdins but not pyocyanin, no growth at 42°C, produces lecithinase
P. putida	Same as *P. fluorescens*	Does not produce pyocyanin or lecthinase, no growth at 42°C
P. cepacia	Endocarditis, septicemia, wound infections	No growth at 42°C
P. stutzeri	Same as *P. fluorescens*	No growth at 42°C
P. maltophila	Found normally in oral cavity; urinary tract infections; except for *P. aeruginosa* most frequently isolated *Pseudomonas* species	Growth at 42°C, lavender-green color on blood agar, only oxidase-negative *Pseudomonas* species
P. mallei	Cause of glanders, a disease of horses occasionally transmitted to humans	Nonmotile, no growth at 42°C
P. pseudomallei	Cause of melioidosis, a pneumonia that may progress to fatal septicemia, in humans	Pyocyanin and pyoverdins not produced, growth at 42°C
Acinetobacter species	Part of indigenous flora of humans; respiratory and urinary tract infections, bacteremia	Coccobacillary, confused with *Neisseria*
Alcaligenes species	Recovered from urinary tract and blood	Oxidase positive, asaccharolytic
Achromobacter species	Isolated from blood, spinal fluid, urine, and respiratory tract	Saccharolytic, oxidase positive
Moraxella species	Isolated from blood, respiratory tract, and urine	Oxidase positive, asaccharolytic
Kingella species	Isolated from blood, nose, joints, and throat	Produces acid from glucose and maltose oxidation
Flavobacterium species	Rarely pathogenic but occasionally cause neonatal meningitis	Nonmotile, proteolytic, prefer cool environment
Eikenella corrodens	Isolated from wounds, respiratory tract, blood, and bone	Nonmotile, asaccharolytic, produces craters in agar
Agrobacterium species	Plant pathogens, but one species, *A. radiobacter*, isolated from wounds and spinal fluid in humans	Motile, oxidase positive

lin in various combinations. An experimental vaccine has been developed for burn patients, who are the most susceptible to infection.

Other genera related to *Pseudomonas* are *Acinetobacter, Alcaligenes, Achromobacter, Moraxella, Kingella, Flavobacterium, Eikenella*, and *Agrobacterium* (see Table 24-2).

QUESTIONS FOR STUDY

1. What characteristics of *Pseudomonas aeruginosa* make it identifiable in wounds?
2. Explain why *P. aeruginosa* is one of the most important pathogens in the hospital environment.
3. Discuss the virulence determinants of *P. aeruginosa*. Despite its virulence determinants what characteristic makes this organism such an important pathogen?
4. How does the exotoxin of *P. aeruginosa* affect host cells?
5. What drugs are effective in disease caused by *P. aeruginosa*?
6. Name two other species of *Pseudomonas* and the diseases with which they are associated.
7. What technique is used in determining the source of *Pseudomonas* infection?

ADDITIONAL READINGS

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25. VIBRIONACEAE

OBJECTIVES

To explain the mechanisms by which *Vibrio cholerae* is able to penetrate the intestinal epithelium and cause diarrhea

To describe the types of disease associated with non-O1 *V. cholerae* and *V. parahaemolyticus*

To explain how gastrointestinal disease caused by species of *Vibrio* can be treated and prevented

Genus *Vibrio*
Vibrio cholerae (O group 1)
Pathogenesis
Cholera Enterotoxin
Epidemiology
Diagnosis
Prevention and Treatment
Vibrio cholerae (non O1)
Vibrio parahaemolyticus
Vibrio vulnificus
Vibrio alginolyticus
Prevention of Gastrointestinal Disease Caused by Vibrios
Genus *Aeromonas*
Genus *Plesiomonas*

Vibrios are straight or curved rods that belong to the family Vibrionaceae. Four genera are included in this family: *Vibrio, Aeromonas*, *Plesiomonas,* and *Photobacterium,* but only *Vibrio* is a major pathogen. Vibrios are gram-negative and are motile by means of a polar flagellum (Fig. 25-1). They demonstrate both respiration and fermentation and, like the Enterobacteriaceae, do not have exacting nutritional requirements. They are found in marine and fresh waters, and some are common causes of intestinal disease in humans.

GENUS *VIBRIO*

There are only a few species of *Vibrio*, the most of important of which is *V. cholerae*, the causative agent of cholera. Other species include *V. parahaemolyticus, V. alginolyticus* and *V. vulnificus*. Each will be discussed separately.

VIBRIO CHOLERAE (O GROUP 1)

V. cholerae (O group 1) is the cause of Asiatic cholera (Fig. 25-2). The O or somatic antigen is important in the serological grouping of the cholera vibrios. The O group 1 serotype consists of two biotypes (biotypes differ in biochemical properties): *cholerae* and *El Tor. El Tor* is responsible for the current pandemic of cholera. The *cholerae* biotype has a greater potential for causing disease, but it is also more susceptible to environmental changes than the *El Tor* biotype.

Pathogenesis

Cholera is spread from person to person by ingestion of contaminated water or uncooked foods, especially fish. *V. cholerae* is very susceptible to gastric secretions, and ingestion of approximately 10^9 vibrios in food or water is required to initiate disease (compare this to previously given numbers of gram-negative bacteria required to cause disease). If the individual's gastric contents have been neutralized by bicarbonate or other antacids, 10^6 vibrios may cause disease. The incubation period is approximately 2–3 days and is followed by a period of vomiting and diarrhea. Ten to twenty-five liters of fluid may be lost during disease. Rapid dehydration is accompanied by a loss of electrolytes such as potassium and bicarbonate. The loss of electrolytes during diarrhea is due to an enterotoxin (see Cholera Enterotoxin). Prostration can occur at any time and is directly related to the amount of fluids lost. Most fatalities occur when the electrolytes have not been replenished. The major causes of death are shock, metabolic acidosis, and renal failure.

There is apparently no complete immunity to reinfection with *V. cholerae* since reinfection does occur in areas where the disease is endemic.

The mechanism by which the cholera vibrios attach to the intestinal epithelium has not been fully elucidated. The mucous gel covering the intestinal epithelial surface is believed to be a normal barrier

Fig. 25-1. Electron micrograph (×40,000 before 40% reduction) of the polar flagellum of *Vibrio parahaemolyticus*. (Courtesy R. Hodgkiss.)

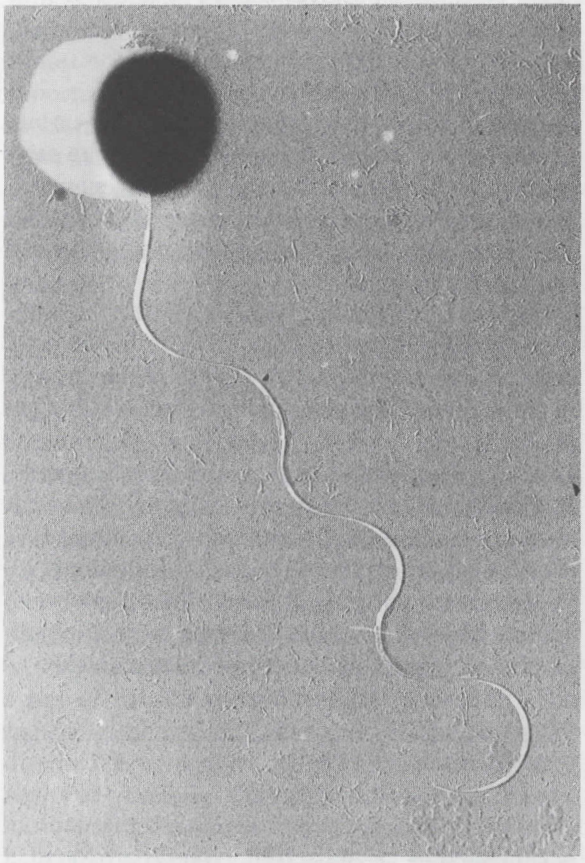
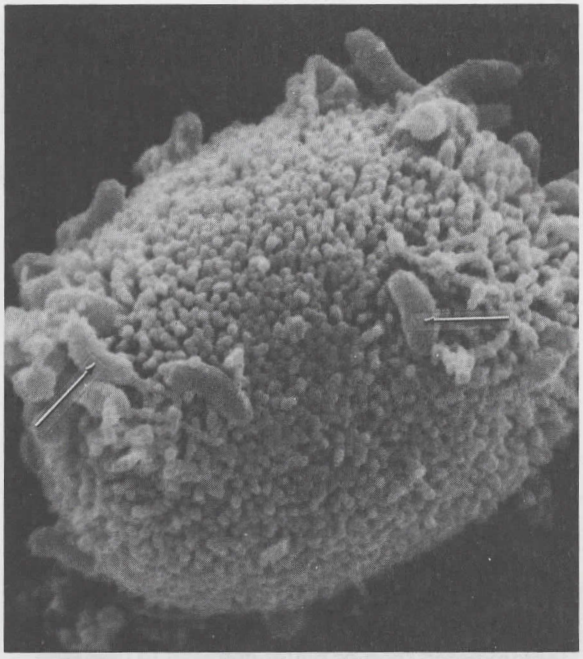


Fig. 25-2. Scanning electron micrograph of *Vibrio cholerae* (*arrows*) attached to the microvilli of a brush border membrane isolated from the small intestine of an infected rabbit. (From R. Freter, *Infect. Immun.* 14:232, 1976.)



to many microorganisms. Vibrios are believed to be able to penetrate the mucous gel because of their motility and chemotactic factors. Once the mucous gel has been penetrated it is believed that a surface component of the vibrio that has protease and hemagglutinating activity enables the organism to attach to the microvilli of host epithelial cells. Once attached the microorganisms can multiply and produce enterotoxins.

CHOLERA ENTEROTOXIN. Cholera enterotoxin (choleragen), which is responsible for the watery diarrhea (rice water stool) associated with disease, is a protein composed of one A subunit and five B subunits. The A subunit is the biologically active portion of the toxin. The B region is important for binding to the cytoplasmic membrane of epithelial cells. The rate at which B subunit binds is apparently dependent on the concentration of a particular glycolipid in the cytoplasmic membrane of the epithelial cell. Cholera enterotoxin affects the activity of adenyl cyclase in the epithelial cell membrane. Adenyl cyclase converts adenosine triphosphate (ATP) to adenosine 3′5′-cyclic phosphate (cyclic AMP). This reaction requires nicotinamide-adenine dinucleotide (NAD) and a cytoplasmic membrane protein. Apparently choleragen hydrolyzes NAD and transfers the adenine diphosphate–ribose (ADP-ribose) portion of NAD to the membrane-bound protein. As ADP-ribose is added there is a concomitant increase in cyclic AMP. The

ADP-ribose transfer reaction is similar to the action of diphtheria toxin (see Chapter 22). Enterotoxigenic strains of *E. coli* produce a heat-labile (LT) enterotoxin that is closely related to choleragen in structure and action. The gene for choleragen synthesis resides in the chromosome, while the enterotoxin gene in *E. coli* is located in a transmissible plasmid.

Epidemiology

Cholera is a disease that occurs worldwide but is seen more frequently in Asia and Africa. Over 36,000 cases of cholera worldwide were reported in 1981. Occasional outbreaks occur in the United States and are confined to the states of Louisiana and Texas. Of 19 cases that occurred in Texas in 1982, 17 were associated with drinking water that had been contaminated with sewage. An outbreak in Kaplan, Louisiana, involved cholera contracted by beach party members' ingesting poorly cooked crabs that were infected with vibrios.

Humans are the primary host of *V. cholerae* and thus the principal source of new cases of cholera. Cholera organisms are harbored by convalescent as well as chronic carriers and are shed into the environment in large numbers. Ingestion of fecally contaminated food or water can lead to disease.

Diagnosis

Isolation of vibrios from fecal specimens is easily accomplished in the laboratory since vibrios are able to tolerate high pH's and are motile. It has been recommended that one enrichment medium and two plating media be used in the isolation and identification procedures. The enrichment medium is alkaline peptone broth (pH 8.4), which suppresses the growth of other intestinal microorganisms. The plating media include tellurite taurocholate gelatin (TTG) agar and thiosulfate citrate bile salts sucrose (TCBS) agar, both of which are selective media. *V. cholerae* produces yellow colonies on TCBS agar. Typical colonies are then streaked on Kligler iron agar and lysine agar. Presumptive identification may be made using *V. cholerae* polyvalent O1 antiserum. Final identification rests on certain biochemical tests that differentiate the species of *Vibrio* and other related species. The *El Tor* biotype is distinguished from the *cholerae* biotype by the former's resistance to polymyxin B, resistance to phage IV, ability to hemolyze sheep red blood cells, and positive Voges-Proskauer test at 22°C.

Prevention and Treatment

Cholera can be prevented by adhering to certain sanitary procedures. There must be adequate sewage treatment and purification of water supplies. Travelers to foreign countries, especially Asia and Africa, are advised against eating raw seafood and uncooked vegetables.

Cholera is self-limiting within a few days if water and electrolytes are replaced. Up to 25 liters of water can be lost during the course of the disease. A replacement fluid consisting of 5 gm NaCl, 4 gm $HaHCO_3$, and 1 gm KCl per liter of sterile water given intravenously has met with great success. Electrolyte solution may also be given orally but must contain glucose to enhance absorption. It is used in milder cases of cholera. Antibiotics, although not required, will lessen the duration and volume of diarrhea and will reduce the vibrio population in the intestinal tract and prevent shedding.

VIBRIO CHOLERAE (NON-O1)

Non-O1 *V. cholerae* are those strains that are not agglutinated by O1 antiserum. Non-O1 are found in sewage; estuarine waters, both polluted and unpolluted; seafoods; and animals. Those strains found in estuarine waters appear to be indigenous free-living forms. They are found in greater numbers during the summer months than during other seasons, but their role in pathogenicity is not known.

Non-O1 *V. cholerae* has been isolated in outbreaks involving gastrointestinal disease but, unlike *V. cholerae* O1, has also been associated with extraintestinal disease. Non-O1 have been isolated from blood and wound infections, primarily in patients already compromised by disease. Non-O1 organisms produce a cholera-like toxin that is believed to be associated with the diarrhea of gas-

trointestinal disease. Most cases are sporadic and not associated with pandemics as classical cholera is. The symptoms of non-O1 disease are similar to those of classical cholera but are less severe. Many outbreaks result from ingestion of mollusks, especially raw oysters. Most disease in the United States occurs in southern coastal waters. The first reported case of non-O1 *V. cholerae* from New England waters was recorded in 1981 and resulted from ingestion of raw clams.

The reservoir of non-O1 *V. cholerae* strains that cause human disease is unknown. Humans may be the reservoir, and occasional carriers have been identified.

VIBRIO PARAHAEMOLYTICUS

V. parahaemolyticus is part of the normal flora of estuarine and other coastal waters throughout the world. Most of the organisms are associated with zooplankton. During winter months these microorganisms remain in the sediment, but as the waters warm the vibrios are released and become attached to minute crustaceans where they proliferate.

V. parahaemolyticus is associated with two types of clinical gastrointestinal syndromes. The most common manifestation of disease is watery diarrhea, accompanied by abdominal cramps, nausea, and vomiting. This disease is self-limiting. A more severe syndrome may be manifested by dehydration, dysentery, and acidosis. This syndrome is usually self-limiting, but occasionally hospitalization is required. No deaths have been recorded in the United States, but some have been recorded in other countries. *V. parahaemolyticus* may also cause extraintestinal disease and has been isolated from wounds and blood.

V. parahaemolyticus strains that cause gastrointestinal illness can hemolyze human or rabbit red blood cells (Kanagawa phenomenon). Nonhemolytic strains are nonpathogenic. Hemolytic strains apparently are equipped with adherence factors that enable them to attach to epithelial cells. How gastrointestinal disease is caused is not known.

Few cases of *V. parahaemolyticus* disease have been recorded in the United States. Most are reported from Asia where disease is usually transmitted by raw or uncooked seafood. Raw food if left at refrigeration temperatures sustains the growth of vibrios. *V. parahaemolyticus* has a maximum generation time of 9 minutes and grows well at temperatures of 25–44°C. Even at less than ideal conditions large numbers of vibrios can be obtained in only a few minutes. The principal reservoir of these vibrios is seafoods and seawater. Carriers do not appear to be important in disease transmission.

VIBRIO VULNIFICUS

V. vulnificus is believed to be part of the normal flora of seawater. Disease caused by this microorganism has been reported in the United States as well as Japan and Belgium. Many cases of disease are extraintestinal and associated with wounds. Such diseases are the result of patient contact with seawater or with saltwater crustaceans. Septicemia has been observed, but the primary focus of infection has not been determined. Most of those incurring disease have some underlying disease or condition that predisposes them to infection.

Patients with wound infections develop rapid swelling around the wound. Infection may also involve surrounding tissue. Large vesicles or bullae with necrosis are also common. Patients with septicemia complain of fever and chills, and most have metastatic cutaneous lesions after the incubation period. These cutaneous lesions are believed to result from hematogenous seeding of the organism. Surgical procedures—debridement, incision, and drainage of wounds—are often required in those with septicemia. Fatality rates are frequently high among those with septicemia because most patients have an underlying disease or condition and the septicemia is recognized too late.

VIBRIO ALGINOLYTICUS

V. alginolyticus is a normal inhabitant of seawater and has a worldwide distribution. The organism is

associated with extraintestinal disease such as wounds or ear infections. Most infections occur after swimming or activities at the seashore. Most lesions require debridement or drainage, but no serious complications occur and most patients respond to antibiotic (erythromycin, tetracycline) therapy. The pathogenic mechanisms of *V. alginolyticus* are not known.

PREVENTION OF GASTROINTESTINAL DISEASE CAUSED BY VIBRIOS

Foods, particularly seafoods, should be cooked at temperatures high enough to kill vibrios and should be protected from cross contamination if they are to be stored before consumption. Storage should be at a temperature either too low (< 4°C) or too high (> 60°C) to permit microbial multiplication.

One should not eat raw seafood or eat any seafood that may be suspected of being fecally contaminated. These measures prevent disease not only by vibrios but by other pathogens such as *Salmonella* species, hepatitis viruses, and other agents of intestinal disease.

GENUS *AEROMONAS*

The genus *Aeromonas* consists of several species with *A. hydrophila* considered the type species. The organism possesses a single polar flagellum. Its habitat is various natural bodies of water, sewage, marine life, and foods. Exposure to soil and water can result in infection by this group of organisms. *Aeromonas* has been associated with gastroenteritis, meningitis, septicemia, and wound infections. Disease by *Aeromonas* occurs primarily in debilitated individuals and has resulted in fatalities. Treatment includes administration of antibiotics such as the aminoglycosides but not penicillins or cephalosporins.

GENUS *PLESIOMONAS*

The genus *Plesiomonas* contains one species, *P. shigelloides*. The organism possesses polar, usually lophotrichous, flagella. It is found in fresh water and in animals. The organism has been isolated from humans—feces, blood, and spinal fluid. It is believed to be the cause of acute gastroenteritis.

SUMMARY

Vibrios are straight or curved gram-negative, aerobic, motile rods that are found in fresh and marine waters. *V. cholerae* O group 1 is the causal agent of cholera and consists of two serotypes, *cholerae* and *El Tor*. Cholera is a gastrointestinal disease caused by an enterotoxin that affects the activity of adenyl cyclase in the cytoplasmic membrane of intestinal epithelium. Cholera is acquired by ingestion of fecally contaminated water or food. Most cases of the disease are observed in Asia and Africa, but some cases occur in the United States, particularly in Louisiana and Texas. The organisms can be detected in stools by microscopic observation and agglutination tests or by plating on selective media. Treatment relies heavily on replenishment of fluids and electrolytes. Non-O1 *V. cholerae* has been isolated in cases of gastrointestinal disease, but the organism can also cause extraintestinal disease. *V. parahaemolyticus* is part of the normal flora of marine waters and can also cause gastrointestinal disease of a mild or severe form. Most cases occur outside the United States and are associated with ingestion of raw seafood, particularly oysters. *V. vulnificus* and *V. alginolyticus* are part of the normal flora of sea water and are associated primarily with wound infections.

The genera *Aeromonas* and *Plesiomonas* are also associated with bodies of water, both fresh and marine. They cause disease that may be gastrointestinal or extraintestinal, particularly in compromised individuals.

QUESTIONS FOR STUDY

1. What is the difference between O group 1 and non–O group 1 *V. cholerae* in terms of morphology? Antigenicity? Disease potential?
2. Why are antibiotics sometimes used in cholera treatment even though electrolyte replenishment is the standard treatment?
3. What is the mechanism of action of cholera enterotoxin? What other toxin does it resemble in activity?
4. If you were looking for species of *Vibrio* where would you look?
5. Under what circumstances might you contract a vibrio-associated disease?
6. How can one prevent gastrointestinal disease caused by vibrios?

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26. GRAM-NEGATIVE COCCOBACILLARY AEROBIC BACTERIA

OBJECTIVES

To understand the types of infections associated with *Hemophilus influenzae* type b

To describe the various stages in whooping cough and the microbial factors believed to be associated with their appearance

To understand the importance and risks of vaccination against whooping cough

To explain the various ways in which the agent of brucellosis may be transmitted to humans

To explain the various ways in which the agent of tularemia may be transmitted to humans

To outline the methods used to treat and prevent *H. influenzae* type b infections, brucellosis, whooping cough, and tularemia

Hemophilus
General Characteristics
Pathogenesis
Encapsulated *Hemophilus influenzae*
Nonencapsulated *Hemophilus influenzae*
Laboratory Diagnosis
Treatment and Prevention
Pathogenic Species Other than *Hemophilus influenzae*
Bordetella
General Characteristics
Pathogenesis
Laboratory Diagnosis
Treatment and Prevention
Pathogenic Species other than *Bordetella pertussis*
Brucella
General Characteristics
Pathogenesis
Laboratory Diagnosis
Treatment and Prevention
Francisella
General Characteristics
Pathogenesis
Laboratory Diagnosis
Treatment and Prevention

The major pathogenic genera belonging to the coccobacillary group of bacteria are *Hemophilus, Bordetella, Brucella*, and *Francisella*. They are the causative agents of meningitis, whooping cough (pertussis), brucellosis (undulant fever), and tularemia, respectively. All are gram-negative, nonmotile coccobacilli that are aerobic or microaerophilic. Their growth on laboratory media is favored by the addition of blood, serum, or elevated concentrations of CO_2. Except for species of *Hemophilus*, most are resistant to penicillin. Many isolates of *Hemophilus* are resistant to ampicillin.

HEMOPHILUS

GENERAL CHARACTERISTICS

Hemophilus influenzae is the predominant pathogenic species of the genus *Hemophilus*. This small gram-negative bacillus is found exclusively in the upper respiratory tract of humans. Other species of *Hemophilus* inhabiting the upper respiratory tract but rarely associated with disease are *H. hemolyticus, H. parainfluenzae, H. parahemolyticus, H. aphrophilus*, and *H. paraphrophilus*. Strains of *H. influenzae* can be divided into two types: nonencapsulated and encapsulated. The nonencapsulated variety is associated with respiratory disease in adults. The encapsulated type is associated with meningitis and other invasive diseases in infants and children. Approximately 8000 to 11,000 cases of meningitis are reported each year in the United States, with the peak incidence between 6 and 7 months of age. Sixteen hundred of these cases are caused by *H. influenzae* in which the mortality is approximately 4 percent.

H. influenzae is fastidious in its growth requirements. It grows best on chocolate agar or enriched media supplemented with two nutritional factors called *X* (hemin) and *V* (nicotinamide-adenine dinucleotide [NAD]). Incubation under 10% CO_2 enhances the growth of most species. Colonies of *H. influenzae* increase in size if they are cultivated in the vicinity of other bacterial colonies—staphylococci, for example. This cooperative effect is called the satellite phenomenon and is due to the production of NAD by the staphylococcal colonies.

PATHOGENESIS

Encapsulated Hemophilus influenzae

Encapsulated strains of *H. influenzae* are primary pathogens, and the most invasive is type b, the

Table 26-1. Causative Agents of Meningitis and the Frequency with Which They Are Associated with Disease

Agent	Frequency[a] (percent)
Hemophilus influenzae[b]	40–50
Neisseria meningitidis	20–25
Streptococcus pneumoniae	20–25
Escherichia coli	4–5
Other species	3–5

[a]The values are approximate and vary from year to year as well as from one community to another.
[b]In children under 4 years of age *H. influenzae* accounts for over 65 percent of the cases of meningitis.

main cause of bacterial meningitis in children under 4 years of age (Table 26-1). By the time children are 4–5 years old nearly 50 percent have acquired *H. influenzae* type b in the nasopharynx. Those colonized by the organism develop resistance to disease by 5 years of age because of antibody. In infants, however, there is no detectable level of antibody so disease leads to serious consequences. Meningitis in this group, even after chemotherapy, leads to serious sequelae such as mental retardation. *H. influenzae* type b infections without meningitis are also common in children and often give rise to other conditions such as pneumonia, bronchitis, otitis media, and epiglottitis. Several studies have revealed that *H. influenzae* type b is one of the major causes of otitis media in children. What is particularly alarming about this statistic is that many of the strains are resistant to ampicillin, the drug most widely prescribed for infection.

H. influenzae type b infections in adults are rare. *Hemophilus* infection in adults occurs more frequently in those who are already compromised by respiratory problems, diabetes, or alcoholism. Infection is manifested as pneumonia and may be caused by encapsulated as well as nonencapsulated strains. *H. influenzae* meningitis has a seasonal distribution with major incidence of disease in the fall and spring. Higher rates of disease occur in blacks, American Indians, and Eskimos.

Resistance to type b infections is believed to be associated with the production of opsonizing antibodies directed against the capsule. The capsular antibodies of type b cross-react with the capsules of pneumococci and meningococci. The common antigenic component of these organisms appears to be polyribophosphate (PRP). When type-specific *H. influenzae* antiserum is mixed with PRP, the bactericidal activity of the antiserum is reduced by 50 percent.

Studies on human volunteers have revealed that injections of purified PRP can stimulate the production of antibodies against *H. influenzae* type b. In 1985 a polysaccharide vaccine against disease caused by *H. influenzae* type b was licensed in the United States.

Nonencapsulated Hemophilus influenzae

The nonencapsulated strains of *H. influenzae* are not invasive but are important secondary pathogens in upper respiratory tract infections. Such infections often occur when the body's defenses have initially been depressed by viral invasion or in cases of chronic bronchitis.

LABORATORY DIAGNOSIS

The presence of contaminating microorganisms in sputum samples often interferes with the preliminary identification of *H. influenzae*. A Gram-stained smear (carbolfuchsin being used as counterstain) of a blood or cerebrospinal fluid specimen, which is normally sterile, can provide a tentative identification of the coccobacillus. Isolation of *H. influenzae* is achieved by culturing clinical specimens on chocolate agar or richer media such as Levinthal's medium. Colonial isolates from the chocolate agar can be identified with type-specific antiserum. *H. influenzae* type b exhibits capsular swelling and agglutination when exposed to type-specific antiserum.

Differentiation of *Hemophilus* species is based on their ability to use accessory growth factors X and V and on their growth response to CO_2. V factor results can be misleading because of carry-over of X factor from media containing blood products. The laboratory separation of *H. influenzae* from *H. parainfluenzae* should be based on the D-aminolevulinic acid utilization test, for which all biotypes of *H. influenzae* are negative while all biotypes of *H. parainfluenzae* are positive.

Several new techniques are available for the detection of *H. influenzae* antigens in the cerebrospinal fluid. The most rapid tests are latex agglutination and coagglutination, in which results can be obtained in 5 to 15 minutes. Thus it is possible to determine the presence of an organism even though one may be incapable of isolating the infectious agent.

TREATMENT AND PREVENTION

Practically all *H. influenzae* infections can be effectively treated with ampicillin, chloramphenicol, or sulfonamide. In 1974, however, several fatalities occurred in children when ampicillin was employed in the treatment of type B infections, and they resulted from the appearance of ampicillin-resistant strains of *H. influenzae*. This resistance is due principally to the production of an R factor–associated beta-lactamase. R factors in *Hemophilus* microorganisms can be transferred by conjugation. It is suggested that sensitivity tests be applied to isolated strains before antibiotic regimens are prescribed. If ampicillin is given and the patient does not immediately respond, other drugs should be substituted. Chloramphenicol has been proposed as an alternative to ampicillin, but, as one might suspect, recent isolates of *H. influenzae* have shown resistance to chloramphenicol as well. For the treatment of meningitis, the combination of streptomycin and sulfisoxazole has proved to be as effective as ampicillin. For the treatment of otitis media, many physicians prefer to use the combination of erythromycin and sulfisoxazole.

Although early treatment of *H. influenzae* infections with antimicrobials is almost 100 percent effective, a few patients who recover have residual central nervous system injury. The *H. influenzae* type b polysaccharide vaccine, recently licensed in the United States, has been shown to be particularly effective in preventing *H. influenzae* type b infection. The vaccine is recommended for all children at 24 months of age and for children 18 months of age who are a high risk group (children who attend day care facilities). The vaccine may also be administered to children between 2 and 5 years of age in whom *H. influenzae* type b disease does occur. The vaccine is not recommended for children under 18 months of age in whom antibody response is very poor. Major research efforts are now directed toward the development of vaccines with immunogenesis in the very young. Several laboratories are developing vaccines in which the capsular polysaccharide of *H. influenzae* is linked with other molecules. The idea is to have a vaccine that will elicit T cell involvement and hence immunogenic memory. One laboratory has mixed PRP with whole *Bordetella pertussis* cells. Preliminary results have shown that together they elicit both IgG and IgM, while PRP alone elicits only IgM antibodies. Another laboratory has treated diphtheria toxoid chemically and linked it with PRP. Studies on this vaccine are now beginning in humans. Outer membrane proteins are also being evaluated as antigens because their purity can be enhanced through the use of monoclonal antibodies.

There is an increased risk of disease in children who come in contact with a person with *H. influenzae* type b meningitis because the organism is transmitted via respiratory droplets. The attack rate is highest in those under 2 years of age. To prevent secondary cases of disease in household contacts rifampin is recommended for all members of the household. Rifampin should not be used in pregnant women because of the drug's teratogenic (capable of causing congenital malformations) potential.

Table 26-2. Characteristics of Species of *Hemophilus* Occasionally Associated with Disease

Species	Disease association	Other characteristics
H. aegyptius	Communicable form of conjunctivitis (pinkeye)	Requires X and V factors; serologically related to *H. influenzae*
H. hemolyticus	Sinusitis, otitis media, and lower respiratory disease	Found normally in upper respiratory tract; beta hemolytic; requires X and V factors
H. parainfluenzae	? Respiratory tract infection and endocarditis	Found normally in respiratory tract; requires V factor
H. ducreyi	Sexually transmitted disease (chancroid)	Fastidious growth requirements; difficult to isolate from lesions; requires X factor
H. paraphrophilus	Infrequent cause of disease but disease is usually severe—endocarditis, septicemia, and meningitis	Does not require V or X factor

Fig. 26-1. Chancroid. Typical lesion on the penis. (From G. Hart. In P. F. Wehrle and F. H. Top, Sr. [Eds.], *Communicable and Infectious Diseases* [9th ed]. St. Louis: Mosby, 1981.)

PATHOGENIC SPECIES OTHER THAN *H. INFLUENZAE*

Several species of *Hemophilus* are occasionally associated with disease (Table 26-2). One of the more important of these is *H. ducreyi*, the causal agent of the sexually transmitted disease *chancroid*. (*Chancroid* refers to an ulcer with a soft base as opposed to the chancre found in syphilis, which has a hard base.) Chancroid is common in Africa and Asia but rare in the United States and other developed countries. The disease is more evident in men, who develop a lesion at the site of infection (Fig. 26-1), than in women. Women are usually asymptomatic and serve as carriers of the disease. The lesions are usually on the penis but in about 50 percent of cases there is inflammation of inguinal lymph nodes and enlargement into buboes. The treatment of choice for this disease is erythromycin. An alternative treatment is sulfamethoxazole and trimethoprim.

BORDETELLA

GENERAL CHARACTERISTICS

Species of *Bordetella* are common inhabitants of the human upper respiratory tract. *B. pertussis* is the causative agent of *whooping cough* (*pertussis*) and is morphologically indistinguishable from other coccobacillary forms. Like *H. influenzae*, *B. pertussis* is fastidious in its cultural requirements. A

special medium called the Bordet-Gengou medium (potato-blood-glycerol) is the primary isolation medium. Other media used contain charcoal, starch, or other agents that absorb inhibitors, such as fatty acids, contained by ordinary laboratory media. Penicillin or methicillin is often added to the medium to inhibit contaminating species of microorganisms that might be present in the clinical isolate. Virulent strains of *B. pertussis* have a capsule (phase I) that is progressively lost on prolonged laboratory passage. Avirulent strains contain no capsule (phase IV) and are less fastidious in their cultural requirements. Because of characteristic differences in surface antigens, *B. pertussis* strains are divided into three serotypes—type 1,2, type 1,2,3, and type 1,3—according to the content of agglutinating factors 1, 2, and 3.

PATHOGENESIS

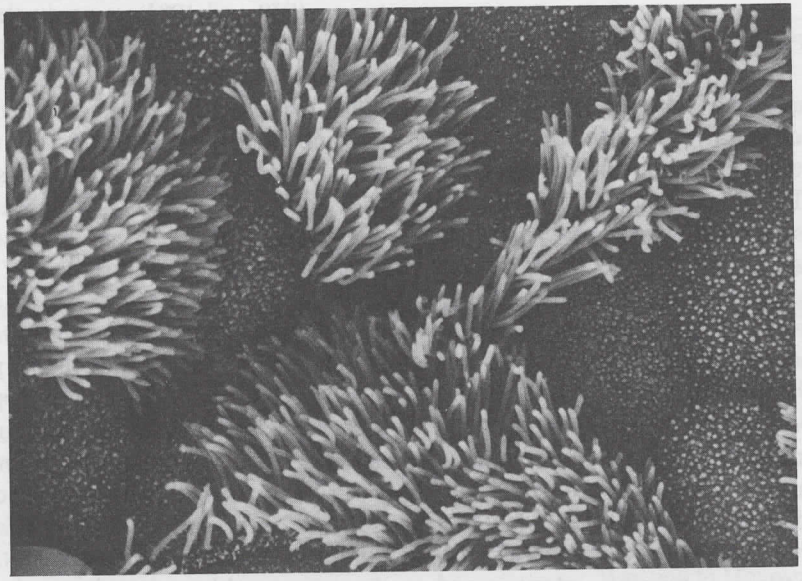
Pertussis is worldwide in distribution with most incidences of disease occurring in the very young. Nearly 80 percent occur in children less than 6 months of age. The first stage of infection is called the *catarrhal* (inflamed mucosal membrane) stage and is characterized by sneezing, coughing, and lacrimation (watery eyes). The catarrhal stage lasts 1–2 weeks and is followed by the *paroxysmal* (sudden attack occurring periodically) stage in which there is an uncontrolled cough that terminates in an inspiratory whoop. (*B. pertussis* does not always produce the characteristic whooping cough.) The paroxysmal stage is the most contagious stage of the disease, and the bacilli can be expelled in the sputum. During this period the bacilli can be seen attached to the epithelium of the bronchi or trachea (Fig. 26-2). *B. pertussis* is the only bacterium to attach exclusively to normal ciliated mucosa. It does not attach to nonciliated epithelial cells. As the disease progresses and more respiratory tissue is involved, blockage of the bronchial passages may take place, leading to lung infection (from pyogenic cocci), convulsions, and sometimes death. The bacilli never invade the bloodstream. (See boxed essay, p. 568.)

Two proteins are believed to be involved in the pathogenesis and immunity of pertussis; pertussis toxin and the filamentous hemagglutinin (FHA). Pertussis toxin is an oligomeric protein composed of a B subunit and A subunit. The B subunit is involved in binding of the toxin to mammalian cell membranes. The A subunit possesses ADP-ribosyltransferase activity, that is, it catalyzes the transfer of the ADP-ribose moiety from NAD to a cell membrane protein. This cell membrane protein is normally involved in controlling cyclic AMP (cAMP). Thus the pertussis toxin directly affects the cAMP content of the host cell (a characteristic similar to cholera toxin, which increases cAMP levels in the cell). Pertussis toxin has a variety of biological effects, such as histamine sensitization, which may be responsible for the cough, and impaired monocyte migration to the site of infection, which may be responsible for maintaining the infection as well as contributing to secondary infections. FHA is believed to be derived from pili and to mediate the attachment of *B. pertussis* to mammalian cells. *B. pertussis* also produces an extracytoplasmic adenylate cyclase that may be involved in virulence. This enzyme is believed to be released during infection and to be internalized by phagocytic cells. The enzyme disrupts normal phagocytic function and thus may help maintain the infection.

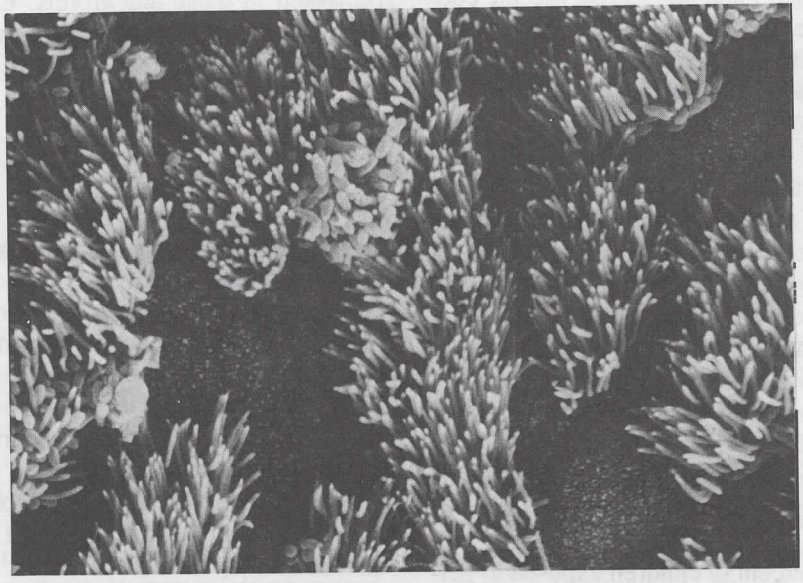
LABORATORY DIAGNOSIS

B. pertussis is not easily isolated from clinical specimens, and diagnosis is usually made on clinical grounds. Leukocytosis is the most common laboratory finding. Diagnosis can be made by identification of the organism in the sputum. The tip of a Nichrome wire is covered with cotton, and the wire is passed through the nose and allowed to touch the nasopharyngeal wall. The wire is removed and the material on it streaked onto a Bordet-Gengou plate containing penicillin and allowed to incubate for at least 72 hours. After the incubation period small, smooth, pearl-like colonies appear. Final identification is achieved by applying antiserum to the co-

Fig. 26-2. *Bordetella pertussis*. A. Scanning electron micrograph (×3000 before 40% reduction) of mouse tracheal epithelium incubated in sterile medium. B. Scanning electron micrograph of mouse tracheal epithelium infected with *B. pertussis*. (From L. B. Opremcak and M. S. Rheins, *Can. J. Microbiol.* 29:415, 1983.)



A



B

lonial isolates. Another approach is through the application of fluorescein-labeled antibody to clinical specimens.

TREATMENT AND PREVENTION

Although whooping cough is often self-limiting, antibiotics such as erythromycin, tetracycline, and chloramphenicol are important in lessening its communicability. These antibiotics are also helpful in reducing the possibility of secondary pneumonia infections. Treatment after the infection is well established has little effect on the course of the illness. Penicillin may be given, however, to reduce secondary infections by pyogenic cocci.

Since the first vaccine for pertussis was administered, the number of cases of whooping cough has decreased from a high of 120,000 per year in 1950 to approximately 2000 in 1983. Active immunization of all children 2–3 months old is highly recommended. The initial vaccination is generally followed by two injections at monthly intervals and booster doses at age 1 and age 5. The vaccine contains killed phase I *B. pertussis* microorganisms and is usually combined with diphtheria and tetanus toxoids (collectively called the DTP). The strain of *B. pertussis* most frequently recovered today from cases of whooping cough is type 1,3. The predominance of this variety is believed to be due to the absence of factor 3 antigen in most vaccine preparations.

The pertussis vaccine normally administered to infants does not confer as long-lasting an immunity as do other vaccines. Probably the reason is the inability of the vaccine to stimulate sufficient concentrations of IgA, which is important in preventing the attachment of *B. pertussis* to ciliated respiratory epithelium. Natural infection with *B. pertussis* stimulates the formation of high concentrations of IgG and IgA.

More than 80 percent of children exposed to pertussis who have received three doses of DTP vaccine will be protected. The number of cases of pertussis in those less than 6 months of age is reduced indirectly because of vaccination of older children. Killed whole cells, however, often cause severe side effects after injection. The most serious side effects are shock and encephalopathy, which usually terminate in death. In the United States between 1979 and 1981 seven deaths were recorded among 1227 cases. These results have frightened many parents to the point that they refuse to have their children vaccinated. Vaccination apathy in Japan and England in the 1970s, for example, has resulted in large epidemics of pertussis in these countries and many pertussis-associated deaths. These results demonstrate the importance of vaccination. There is a need for a new type of vaccine. A new vaccine was developed in Japan and has been in use in that country since 1982. The Japanese vaccine is composed of two cellular proteins that exhibit hemagglutinin activity. It appears to be protective without inducing serious side effects; however, the results are only preliminary.

Anyone contracting whooping cough should be isolated from children. *B. pertussis* can still be harbored by older children and adults, and therein lies the major problem since immunization should not be risked in these age groups. Erythromycin has been suggested as chemoprophylaxis for adults who come in contact with pertussis patients.

PATHOGENIC SPECIES OTHER THAN *BORDETELLA PERTUSSIS*

Other *Bordetella* species that may be found in the upper respiratory tract are *B. parapertussis* and *B. bronchiseptica*. *B. parapertussis* causes a mild form of whooping cough in humans and can be differentiated from *B. pertussis* by the former's ability to hydrolyze urea and use citrate as a sole source of carbon. *B. bronchiseptica* is found more often in animals or persons who come in contact with domestic animals. Differentiation of the species of *Bordetella* can be accomplished by certain biochemical tests (Table 26-3).

Table 26-3. Characteristics Differentiating Species of *Bordetella*

Test	*B. pertussis*	*B. parapertussis*	*B. bronchiseptica*
Pigment production	−	+	−
Urease production	−	+	+
Oxidase production	+	−	+
Nitrate reduction	−	−	+
Motility	−	−	+
Citrate utilization	−	+	+

Table 26-4. Characteristics Used in Differentiating the Three Major Species of *Brucella*

		Growth in Presence of		
Species	H_2S Production	Basic Fuchsin 1 : 25,000	Thionin 1 : 50,000	Methyl Violet 1 : 50,000
B. abortus	+ +	+	−	+
B. melitensis	+	+	+	+
B. suis	+ + +	−	+	−

			Reaction to Monospecific Serum of	
Species	CO_2 Requirement	Urease Activity	*B. abortus*	*B. melitensis*
B. abortus	+	−	+	−
B. melitensis	−	−	−	+
B. suis	−	+	+	−

BRUCELLA

GENERAL CHARACTERISTICS

The genus *Brucella* is composed of species that are primarily pathogenic to animals and that secondarily infect humans. There are three principal species: *B. melitensis*, which infects goats and sheep; *B. abortus*, cattle; and *B. suis*, swine. All three species can infect humans, and the disease produced is referred to as *brucellosis*. Human infection caused by brucellae is sometimes called *undulant fever*.

The brucellae are nonmotile coccobacilli capable of producing capsules. They are difficult to differentiate, and species separation is based on sensitivity to dyes, H_2S production, urea hydrolysis, and agglutination (Table 26-4).

PATHOGENESIS

Brucellosis is common in areas where raw unpasteurized milk is consumed from infected cows and goats. It also occurs among farmers, veterinary surgeons, and slaughterhouse personnel who are in contact with infected animals. Most infections remain asymptomatic. Recent outbreaks in the United States have been associated with ingestion of cheese made from unpasteurized goat milk. Raw milk cheese is also frequently contaminated with other potential pathogens including *Salmonella*,

PERTUSSIS SURVEILLANCE, 1979–1981

In the 3-year period 1979–1981, the supplementary surveillance system received reports of 1227 cases from 42 states. The largest numbers of cases were reported by Indiana (185), Washington (125), and New York (105).

Analysis of the data from this supplementary system indicates that 62 percent of the cases involved children < 1 year of age and that 79 percent of these cases were among children < 6 months of age. The high percentage of cases found among < 1-year-olds by this supplementary system is similar to the 57 percent of pertussis cases in that age group reported in the *MMWR* in 1980.

Of 479 reported pertussis patients aged 6 months to 9 years with known vaccination status, 33 percent were not vaccinated with DTP before becoming ill and 60 percent had received < 3 doses.

Findings for the patients on whom clinical information was available showed that 72 percent had a whoop and 41 percent had apnea. Pneumonia was reported as a complication for 29 percent of the patients. The frequency of pneumonia was highest (34 percent) among infants < 6 months of age. For patients < 4 years of age, the likelihood of pneumonia was inversely related to the number of DTP doses.

Seizures were reported for 51 (4 percent) of 1227 patients; 29 (57 percent) of these patients were < 6 months of age. Among patients < 4 years of age, the likelihood of developing seizures was also inversely related to the number of DTP doses received; however, the difference was not statistically significant. Encephalopathy was associated with 0.4 percent of cases. All patients reported to have encephalopathy were < 1 year of age.

Fifty-eight percent of all reported patients were hospitalized, including 80 percent of infants < 6 months of age, 60 percent of those 6–11 months of age, and 35 percent of those 1–4 years of age. Relatively few patients > 4 years of age were hospitalized. The proportion of patients hospitalized was inversely related to the number of doses of DTP vaccine received; the highest proportion of patients hospitalized occurred among children who had received < 3 doses of DTP vaccine.

Seven deaths (0.6 percent) associated with pertussis were reported. All deaths occurred among hospitalized patients who were < 1 year of age, had not been vaccinated, and had pneumonia.

From *MMWR* 31:333, 1982.

Escherichia coli, Mycobacterium tuberculosis, and *Campylobacter fetus* subspecies *jejuni*.

In the United States approximately 200 cases of brucellosis are reported each year. Most infections occur among workers in the meat-processing and livestock industries. Accidental infection among veterinarians and laboratory workers represents a small but important incidence. Domestic swine are the major source of infection, and *B. suis* is the most commonly isolated species. Recently there have been recorded cases of brucellosis resulting from contact with infected dogs (*B. canis* the causal agent). *B. canis* infections have been associated with kennel epidemics, particularly among beagles. Infection is characterized by embryonic deaths or by abortions at about the fiftieth day of gestation.

Brucella infection in humans is the result of ingestion of microorganisms in contaminated cow's or goat's milk or contact with infected animal carcasses. (See boxed essay, p. 570.) The organisms gain entrance to the body through abraded skin or mucous membranes of the intestinal or respiratory tract. They do not persist very long at the site of entry and are ingested by neutrophils, quickly settling in regional lymph nodes. In the intracellular environment of the neutrophil the bacilli reproduce and subsequently lyse the cell. The released bacteria at this stage may be destroyed by phagocytes, or they may overcome the host defenses and be transported within macrophages via the blood to many areas of the body. The bacilli localize in tissue (liver, bone, spleen), where granulomas similar to those found in tuberculosis are produced. Viable bacilli may persist for several months in the granulomas, giving rise to acute or chronic symptoms. For this reason the incubation period in brucellosis may be very long. The acute stage, which usually appears approximately 1–2 weeks after infection, is characterized by fever and enlargement of the lymph nodes, spleen, and liver. The temperature during the acute phase ranges between 101 and 104°F and may continue for several weeks with intermittent remissions (undulant fever). The chronic stage is generally associated with a hypersensitivity in which there may be swelling of the joints and persistence of a low-grade fever.

LABORATORY DIAGNOSIS

Diagnosis of brucellosis is difficult because the disease is generally a chronic one accompanied by fever and few symptoms. The microorganisms are best recovered from the blood during the fever stage. Blood cultures often become negative after the first few weeks of infection on account of development of antibodies by the patient.

The clinical specimens can be cultivated on trypticase soy or liver infusion agar in an atmosphere of 2 to 10% CO_2. Blood cultures should be retained for 4–6 weeks before being discarded as negative. Isolation from contaminated milk is carried out by means of selective media containing horse serum, dextrose, and various bacteriostatic dyes and antibiotics.

Agglutination tests on the patient's serum using heat-killed *Brucella* antigen are the most reliable serological tests. Persons immunized against cholera, however, also have agglutinins for *Brucella*. An indirect fluorescent antibody test has likewise been used for detecting *Brucella* antibodies in human sera. The enzymed-linked immunosorbent assay (ELISA) test is being used for surveillance of *Brucella* antibodies in cattle.

TREATMENT AND PREVENTION

B. abortus infection in cattle is responsible for bovine abortion. The pathogen exhibits a tissue tropism by virtue of its requirement for the sugar erythrose, which is abundant in the placenta of the cow. Mass testing, slaughter of infected cattle, and vaccination of heifers have reduced the infection rate of cattle in the United States to less than 5 percent. Infection in swine (*B. suis*), however, remains at a very high level, and disease is highest among personnel in slaughterhouses.

A satisfactory vaccine for humans has not yet been produced.

Because of the intracellular environment of the brucellae in the infected host, effective treatment is often difficult. Brucellae are susceptible to ampicil-

BRUCELLOSIS—TEXAS

On April 7, 1983, a case of possible brucellosis was reported to the city of Houston, Texas, Health Department's Bureau of Epidemiology. Three weeks later, 12 suspected cases had been reported, and by July 19, 29 cases were identified. Six of the cases were reported by physicians and nurses, and the remainder were discovered during epidemiologic investigation in the community.

The patients ranged in age from 2–81 years. All were Mexican emigrants living in northeast Houston. Twenty-eight of the twenty-nine patients reported eating goat cheese (*queso blanco*) before the onset of symptoms. Twenty-three patients reported purchasing cheese from neighborhood weekend vendors selling the product from their motor vehicles.

The cheese was reportedly produced in Linares, Mexico, and laboratory analysis determined that it was unpasteurized; all attempts to isolate *Brucella* spp. from the cheese were unsuccessful.

In the 14-year period 1965–1978, 3316 cases of brucellosis were reported to the Centers for Disease Control (CDC) by state health departments. Unpasteurized (raw) dairy products accounted for 268 (8 percent) of these cases; 127 (47 percent) of the 268 cases were associated with raw dairy products from Mexico, predominantly fresh cheese made from unpasteurized goat milk, as in the present outbreak. Similar outbreaks have been reported previously from Colorado and Texas.

An additional 50 (19 percent) of the 268 raw dairy product–associated cases were traced to ingestion of products from countries in the Mediterranean basin, Far East, Middle East, and South America. Most were sporadic events involving individual American travelers or foreign visitors to the United States who consumed dairy products acquired abroad; however, outbreaks of multiple cases have occurred among members of tour groups to countries bordering the Mediterranean Sea. Because these individuals frequently have no vocational or avocational exposure to *Brucella* spp, other more common causes of fever are generally ruled out before a diagnosis of brucellosis is entertained, thereby delaying appropriate therapy. Countries reporting more than 1000 human cases of brucellosis per year in their indigenous populations include Argentina, Greece, Iran, Italy, Mexico, Peru, and Spain. The remaining 91 (34 percent) raw dairy product–associated human brucellosis cases were linked to unpasteurized milk or cheese produced in the United States.

Unpasteurized dairy products have been associated with group C streptococcal infections in New Mexico. Raw milk cheese contaminated with staphylococcal enterotoxin is reported to be widespread. In addition, salmonellae, which are readily killed by pasteurization, have repeatedly been shown to survive the cheese-making process. Other organisms that have caused cheese-associated diseases or have been shown to survive the cheese-making process include *Escherichia coli, Mycobacterium tuberculosis*, and *Campylobacter fetus* subspecies *jejuni*.

From *MMWR* 32:548, 1983.

lin, streptomycin, and tetracycline. Tetracyclines are the first choice in treatment. Successful treatment is best obtained during the acute stage of the disease. During chronic infections antibiotics are unsatisfactory and relapses are common.

FRANCISELLA

GENERAL CHARACTERISTICS

The most important species of the genus *Francisella* (formerly *Pasteurella*), is *F. tularensis*, the causative agent of *tularemia*. The genus name honors Dr. Francis while the species name indicates Tulare county in the state of California, where the disease was first described. Tularemia is a disease of rodents, particularly rabbits, and is transmissible to human beings. Humans can become infected by direct contact with infected animals through handling or ingestion, indirectly by the bite of insect vectors such as ticks or deerflies, or by inhalation of contaminated aerosols. Approximately 200 cases of tularemia are recorded each year in the United States.

F. tularensis is a gram-negative, nonmotile bacillus that can produce a capsule in vivo. It is highly pleomorphic in culture and can resemble filamentous, coccal, or bacillary forms. It is fastidious in its culture requirements. Reducing agents such as cystine or thioglycolate must be supplied in the medium if it is to achieve maximum growth.

PATHOGENESIS

Two types of *F. tularensis*, each with different characteristics, have been described. Type A strains are more virulent than type B strains, cause 5 to 7 percent mortality in untreated cases, and are usually associated with rabbits and tick vectors. Type B strains are less virulent, rarely cause death in untreated cases, and are associated primarily with rodents, such as muskrats. Type A strains are found only in North America where they cause 80 to 90 percent of the reported cases. Type A strains can be differentiated biochemically from type B strains by the former's ability to ferment glycerol and possession of the enzyme citrulline ureidase.

Most cases of tularemia result from contact with infected animal tissue (skinning infected animals such as rabbits—so-called rabbit fever) or from eating infected meat. (See boxed essay, p. 572.) One-third of the cases of tularemia in the United States are the consequence of bites of ticks or deerflies carrying the infectious bacilli. Laboratory personnel caring for animals can contract tularemia through contaminated aerosols generated by sneezing animals.

The incubation period in tularemia is from 2–10 days and is coupled with fever and headache. From the original site of infection the bacilli migrate to regional lymph nodes, causing their enlargement. Like the brucellae, *F. tularensis* can persist in the intracellular environment of the phagocyte. No explanation has been given for the microorganism's ability to survive phagocytosis. During their intracellular sojourn the bacilli can enter the bloodstream and infect a number of organs, including the spleen, liver, and lungs, producing granulomas.

The most common clinical type of tularemia is the ulceroglandular variety. The primary lesion, usually found on the hands and arms, becomes ulcerated in 6–8 days (Fig. 26-3). During this period there will be fever and involvement of local lymph nodes. The lesions may take 2–5 weeks to heal.

Accidental contamination of the eye can result in oculoglandular tularemia, in which there is ulceration of the conjunctiva.

The most severe form of tularemia is the pneumonic variety, usually contracted through contaminated aerosols. If left untreated, this clinical type of tularemia can be fatal to 30 percent of persons infected. Recovery from all three forms of tularemia confers permanent immunity.

LABORATORY DIAGNOSIS

The risk to laboratory personnel of infection by *F. tularensis* is so high that tularemia is diagnosed on

TULAREMIA ASSOCIATED WITH DOMESTIC CATS—GEORGIA

On May 17, 1981, a 32-year-old man living in rural southeast Georgia had onset of fever and noted a papule on the dorsum of the right thumb. The following day, trimethoprim-sulfamethoxazole was prescribed. On May 21, therapy was changed to ampicillin because of a persistent fever that was thought to be caused by sinusitis.

The patient was hospitalized on May 26 with a temperature of 101°F (38.3°C). A necrotic, ulcerating lesion, with associated ascending lymphangitis and tendon lymphadenitis, was present on the dorsum of his thumb. Findings of the remainder of the physical examination were normal. The white blood cell count was 12,900/mm^3, with a differential count of 56 segmented neutrophils, 7 band forms, 24 lymphocytes, 4 atypical lymphocytes, and 9 monocytes. A Gram stain of lesion exudate, as well as aerobic and anaerobic bacterial cultures on routine media (which did not include the glucose-cysteine blood agar usually required for the growth of *Francisella tularensis*), revealed no organisms. A biopsy of the ulcer showed skin necrosis.

Because a consulting physician suggested a diagnosis of tularemia, streptomycin, 1 gram intramuscularly every 12 hours, was administered; 2 days later, the dose was lowered to 500 mg every 12 hours and continued for an additional 10 days. The patient recovered uneventfully. Tests of acute- and convalescent-phase serum specimens, using the tube agglutination technique for detection of *F. tularensis* antibody, revealed a rise in titer from 20 on May 29 to 2560 on June 10.

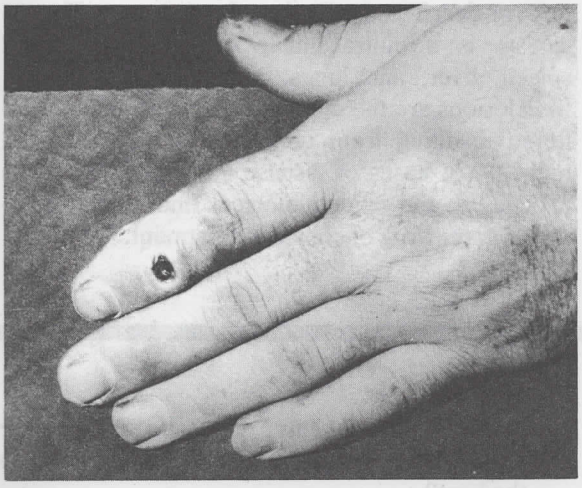
In the 2 weeks before onset of the patient's illness, his three Siamese cats had fever and became anorectic and listless. The family veterinarian prescribed penicillin and streptomycin on May 8, and the patient treated the cats at home without receiving any injuries that he could recall. Despite therapy, two of the cats died on May 13, and the third, noted by the veterinarian to be icteric shortly before death, died May 17. Two cats were necropsied; the livers of both animals contained numerous discrete white spots 3 mm in diameter. On microscopic examination these foci, as well as others in the spleen, were found to be areas of acute inflammation containing coccobacillary organisms. The organisms were immunofluorescence-positive for *F. tularensis*, but cultures prepared after the tissues had been frozen were negative.

Cases of human tularemia associated with cats are uncommon. Humans may acquire the disease by the bite or scratch of a cat, which, though infected, may show no signs of clinical illness. Bite transmission is more commonly reported and probably reflects the presence of the organism in the mouths of cats after they have eaten or mouthed infected animals, usually rodents or rabbits. However, in the absence of a known scratch, bite, or break in the patient's skin, exposure to a cat with an ulcer has also been reported as a source of infection. While *F. tularensis* has been reported to penetrate

unbroken skin of guinea pigs and mice, it seems plausible that the organism enters through minute abrasions on the skin. This is the most likely route of infection in the Georgia case, where there was no history of a bite or scratch. Although lymphangitis was noted in this case, involvement of the regional lymphatic vessels is rare in tularemia and usually manifests as firm, nontender nodules.

From *MMWR* 31:39, 1982.

Fig. 26-3. Typical cutaneous lesion of tularemia. (Courtesy Armed Forces Institute of Pathology, AFIP 83587-2.)



the basis of clinical findings and the patient's history of recent animal or tick exposure. In addition, it is very difficult to isolate this very fastidious organism. *F. tularensis* specimens from blood, sputum, or conjunctiva must be cultured on media rich in cystine or other reducing agents. The most popular medium is a blood-cystine-dextrose agar. The colonies that appear 3–5 days after incubation of the specimen are minute and transparent. Identification depends on staining with fluorescein-conjugated antibody or agglutination by specific antisera. The ELISA has also been developed for diagnosis of tularemia. The advantage of the ELISA is that the first serum sample from the patient can be diagnosed for tularemia. In agglutination tests two serum samples are usually required.

TREATMENT AND PREVENTION

Streptomycin is the most effective drug in the treatment of tularemia, although chloramphenicol and tetracycline are sometimes employed. Relapses are more common when tetracyclines or chloramphenicol are used because they fail to penetrate the intracellular environment of the bacilli in any adequate concentration. An attenuated vaccine is available for use by laboratory personnel who are continually in contact with potentially infected animals.

SUMMARY

Hemophilus, Bordetella, Brucella, and *Francisella* make up the group called the gram-negative coccobacillary bacteria. *Hemophilus influenzae* is found in the upper respiratory tract of humans. *H. influenzae* type b is an important pathogen for humans and is the most frequent cause of meningitis, especially in children under 4 years of age. Pathogenicity is associated with the capsule of this organism. The pathogen can be grown on laboratory media, and identification of colonies is made by applica-

tion of type-specific antiserum. Ampicillin, chloramphenicol, and sulfonamide are useful in treatment of disease. *H. aegyptius* is the causative agent of pinkeye, a mild conjunctivitis. *H. ducreyi* is involved in a sexually transmitted disease called chancroid.

Bordetella pertussis is found in the upper respiratory tract of humans and is the causal agent of whooping cough, a highly contagious disease. Virulent strains produce a large capsule, which is lost on serial passage in the laboratory. The disease remains localized in respiratory tissue and may eventually lead to blockage of the bronchial passages. Laboratory diagnosis relies on isolation of a respiratory specimen, which is streaked on a special medium called Bordet-Gengou. Colonies are stained and identified. Fluorescent antibody applied to specimens can also be used for identification. A pertussis vaccine, part of the DTP vaccine-toxoid complex, is available to the population. Antibiotics do not cure the disease, but they reduce the chances of secondary infections.

Brucella organisms cause diseases referred to as brucelloses. The species involved in disease are *B. abortus, B. melitensis*, and *B. suis*. These species are inhabitants of animals and are secondarily transmitted to humans. Most diseases occur in slaughterhouses where contact with contaminated animal carcasses can lead to disease. Consumption of raw milk is a frequent means of transmission of the microorganism in other countries. Brucellae are intracellular parasites and are difficult to eradicate; thus they are associated with chronic disease. Bacilli localize in liver, bone, and spleen where they produce granulomas. Diagnosis of disease is difficult, and the most reliable technique is based on serological tests. Ampicillin, streptomycin, and tetracyclines are useful only in the acute phase of the disease. A vaccine is available only for cattle.

Francisella tularensis causes the disease tularemia, which is prevalent in rodents and rabbits and is transmitted to humans. Disease is acquired by handling or eating infected rabbits or by bites of ticks or deerflies that harbor the pathogen. The organism is an intracellular parasite and infects spleen, liver, and lungs, producing granulomas. Skin lesions are the most frequent clinical manifestation resulting from contact with contaminated meat. Most cases of disease are diagnosed based on clinical and epidemiological findings. Streptomycin is the drug of choice in treatment.

QUESTIONS FOR STUDY

1. Is *H. influenzae* type b found in infants as well as adults? Why are infants so severely affected by disease?
2. What innovations are being used in the preparation of pertussis vaccine? What are some problems associated with the currently used vaccine?
3. What type of tissue is affected by *H. influenzae, B. pertussis, Brucella abortus*, and *Francisella tularensis*?
4. What diseases are caused by *H. ducreyi*? *H. aegyptius*?
5. How is brucellosis usually contracted in the United States? In foreign countries?
6. What do *Brucella* and *Francisella* species have in common during their multiplication cycle in the host?
7. What type of tularemia is most frequently encountered in the United States? What are its clinical manifestations?
8. Why is brucellosis a chronic disease?
9. Speculate as to why antibiotics do not affect the course of whooping cough. Why are antibiotics administered during this disease?
10. What organism is the most frequent cause of bacterial meningitis in the United States? The second most frequent cause?

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27. MYCOBACTERIA

OBJECTIVES

To describe the stages from inhalation of the tubercle bacillus to the formation of a tubercle

To outline the drugs used in the treatment and prevention of tuberculosis

To understand the immune response associated with tuberculosis and its relationship to vaccination and resistance to infection

To briefly describe the techniques used to identify mycobacteria in the laboratory

To explain the difference between tuberculoid and lepromatous leprosy

Mycobacterium Tuberculosis
General Characteristics
Pathogenesis
Immunity
Tuberculin Tests
Laboratory Diagnosis
Epidemiology
Treatment and Prevention
Nontuberculous Mycobacterioses
Pathogenesis
Laboratory Diagnosis, Treatment, and Prevention
Mycobacterium leprae
General Characteristics
Pathogenesis
Laboratory Diagnosis, Treatment, and Prevention

The mycobacteria are ubiquitous microorganisms that can be readily found in animals and humans. Two diseases of medical importance, tuberculosis (TB) and leprosy, are caused by *Mycobacterium* species. Diseases caused by the mycobacteria are called *mycobacterioses*. The causative agents of tuberculosis in humans are *M. tuberculosis*, the human strain, and *M. bovis*, the strain found in cattle. Tuberculosis is particularly injurious to lung tissue and is widespread throughout the world. Although control measures have reduced the mortality in the United States and other developed countries, as many as 4 to 5 million people each year die of TB. Leprosy, a disease that produces lesions of the skin and nerves, is caused by *M. leprae*. This disfiguring ailment is found in Asia, islands of the South Pacific, and other tropical areas. There are approximately 5 million lepers in the world; few deaths, however, are directly related to the disease.

MYCOBACTERIUM TUBERCULOSIS

GENERAL CHARACTERISTICS

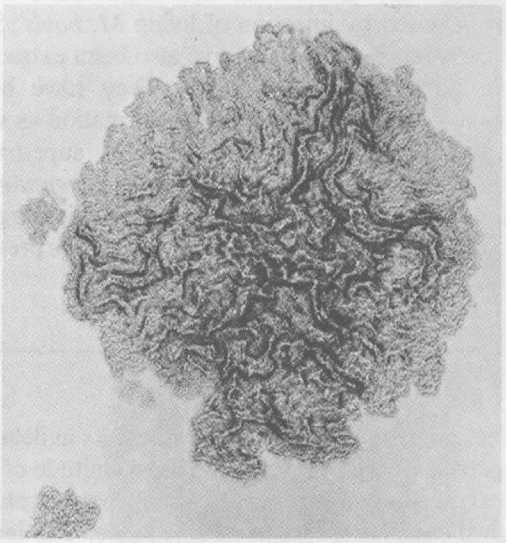
The mycobacteria are aerobic, nonsporeforming, nonmotile bacilli that measure 3.0 by 0.47 μm. They are difficult to stain and can resist decolorization with acid alcohol when they are stained with basic dyes. Because of this property, they have been referred to as the *acid-fast bacilli*. The best known acid-fast staining procedure is the Ziehl-Neelsen technique. The ability of the bacilli to resist decolorization is related to the abnormally high lipid content of the cell wall. The lipid content may approach 60 percent of the cell weight.

Many media used to cultivate mycobacteria in the laboratory consist of a basal medium containing egg or egg yolk. One such medium is called the Löwenstein-Jensen medium. It incorporates malachite green to inhibit the growth of bacterial contaminants. Depending on the mycobacterial species, colonies may not be evident on this medium for 2–6 weeks after inoculation. Most mycobacteria grow on this medium. The colonies produced by *M. tuberculosis* are raised, wrinkled, buff colored, and not easily emulsified. *M. bovis* also grows on this medium; its colonies, however, are low, small, smooth, and easily emulsified. On special transparent media such as 7H10 agar, the roughness of the colonies of *M. tuberculosis* can be observed microscopically as a pattern of serpentine cords (Fig. 27-1). This characteristic is due to the high lipid content of the microorganisms. The "cord factor" can be extracted and is associated with virulence of most pathogenic mycobacteria. Some species of *Mycobacterium*, particularly the nonpathogens, produce pigments (are chromogenic). These pigments may or may not require light for their formation.

PATHOGENESIS

Tuberculosis, like syphilis, is an infectious granulomatous disease that may last the lifetime of the

Fig. 27-1. Serpentine cord colony of *Mycobacterium tuberculosis*. (From E. H. Runyon, A. G. Karlson, G. P. Kubica, and L. G. Wayne. In E. H. Lennette, A. Balows, and W. J. Hausler, Jr., and J. P. Truant [Eds.], *Manual of Clinical Microbiology* [3rd ed.]. Washington, DC: American Society for Microbiology, 1980.)



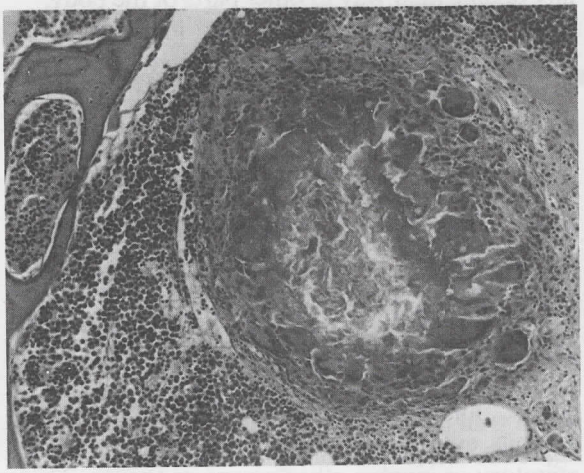
patient, that is, it is a chronic disease. The TB bacillus can infect any organ of the body but is usually associated with the lungs. Because of current control measures on dairy cattle, *M. bovis* is not a major factor in disease in the developed countries of the world, but *M. tuberculosis* is still an important pathogen even in developed countries such as the United States. Infection occurs mostly by direct contact with the infecting bacilli via droplet nuclei, ingestion of contaminated milk, or inhalation of contaminated dust particles. The extent of the disease is not related to the virulence of the infecting bacilli but to such factors as the immunity of the host, the hypersensitivity of the host, and the infecting dose of bacilli.

First infections are more prevalent in children than in adults. After inhalation of the bacilli, a lesion develops in the alveoli of one lung. This is followed by an inflammatory response in which there is an accumulation of neutrophils and macrophages. The bacilli are often carried to regional lymph nodes. Bacilli in the lung and lymph tissue lesions are ingested by macrophages and continue to multiply. The primary lesion may heal, with resorption of the exudate and destruction of the bacilli, but many bacilli survive. Surviving bacilli may reach the thoracic duct and bloodstream, eventually finding their way to other parts of the body.

At 3–12 weeks after the initial infection an immunological response can be detected. T cells and B cells appear at the lesion and respond to the appearance of the mycobacteria. The lesions, whether in the lung or other organs and tissues, are walled off in up to 85 to 95 percent of those infected. These sites of infection, however, still retain viable bacilli, which can later be reactivated. This class of individuals show no symptoms of disease although they do exhibit a delayed hypersensitivity. The remaining 5 to 15 percent show foci of infection that result in a specific pathological response, and bacilli proliferate, that is, the patient exhibits evidence of tuberculosis that may be respiratory or involve other organs or tissues. These manifestations of disease are referred to as *primary tuberculosis*. Microorganisms that persist and multiply at the site of infection are joined by mononuclear cells, tissue macrophages, and other cells. Dense connective tissue surrounds this site of cellular accumulation, which is referred to as a *tubercle* (Fig. 27-2). The tubercle observed microscopically appears as a granular nodule (granuloma) and represents the individual's hypersensitive response to the invading bacilli.

If large numbers of bacilli are produced in the tubercle, the number of neutrophils at the lesion will also increase. The release of lysosomal enzymes destroys not only the bacilli but the tubercle as well, and so functional host tissue is destroyed. A coagulated mass of host cells and bacilli referred to as *caseation necrosis* thus appears. The caseous lesion may heal by calcification with bacilli remaining viable within it for years. The calcification of the lesion permits its radiographic visualization. If the caseous lesion ruptures, viable bacilli can be disseminated by a systemic or pulmonary vessel to other organs of the body to form tubercles. This type of disseminated tuberculosis is called *miliary*

Fig. 27-2. Histology of a tubercle (× 160 before 35% reduction). Tubercle is in the bone marrow of wild turkey. Central necrosis can be observed with multinucleated giant cells surrounding it. A well-defined capsule delineates the tubercle. (From C. O. Thoen, A. G. Karlson, and E. M. Himes, *Rev. Infect. Dis.* 3:960, 1981.)



tuberculosis (Fig. 27-3). If the caseous lesion ruptures, viable bacilli are carried to a bronchus, where they can infect healthy lung tissue. Bacilli can be aspirated into the lower portions of both lungs (Fig. 27-3). The bacilli may also be aspirated in the sputum. In some individuals the tuberculous cavity becomes encapsulated by fibrous walls of connective tissue and persists for years, constantly draining bacilli into the bronchi.

Even when the disease has apparently terminated, reinfection may occur by (1) activation of the bacilli that survived the primary infection or (2) inhalation or ingestion of new bacilli from the environment. This manifestation of disease is called *reactivation tuberculosis* and occurs primarily in the elderly. Malnutrition, diabetes mellitus, prolonged corticosteroid therapy, and chronic alcoholism are factors that predispose the patient to reactivation of tuberculosis. A strong tuberculin skin sensitivity is an indicator that an infected individual has a greater probability of developing active disease.

A microbial factor associated with virulence, as discussed earlier, is believed to be the cord factor, a glycolipid (Fig. 27-4) that has been extracted and demonstrated to exhibit a variety of properties. Cord factor inhibits the migration of leukocytes, and organisms lacking it have reduced virulence. Cord factor injected into mice causes the formation of pulmonary granulomas indistinguishable from those elicited by injection of living *M. bovis*. Sulfur-containing glycolipids have also been extracted from virulent tubercle bacilli. They have been shown to inhibit phagolysosome formation as well as mitochondrial phosphorylation. A superoxide dismutase has been isolated from *M. tuberculosis*. This enzyme presumably enables the microorganism to resist destruction by superoxide ions present in phagocytic cells.

IMMUNITY

Infection by the tubercle bacillus results in delayed hypersensitivity by the host. The magnitude of the hypersensitivity, however, cannot be correlated with the degree of acquired resistance. The intensity of the hypersensitivity response is directly proportional to the amount of mycobacterial antigen present in the infected host. Large numbers of tubercle bacilli in tissue are associated with a highly destructive lesion because of the sensitivity to mycobacterial antigens (tuberculin). This hypersensitivity does not prevent the bacilli from multiplying in tissue.

Resistance to tuberculosis is related to the ability of macrophages to kill the bacilli or to prevent them from multiplying. An individual can remain infected for many years without demonstrating overt disease if macrophages can prevent the bacilli from multiplying.

Tuberculin Tests

Purified tuberculin protein derivative (PPD) is used in skin sensitivity tests. When injected intracutaneously (*Mantoux test*) into hypersensitive individuals, tuberculin evokes an intense inflammatory reaction (delayed hypersensitivity) at the site of injection. The activity of PPD is standardized into

Fig. 27-3. Bilateral tuberculosis. Caseous necrosis is observed in each lower lobe (*arrows*) as well as in hilar and tracheobronchial nodes. Advanced miliary dissemination can be seen (as white speckled areas) throughout both lungs. (From W. Giese. In E. Kaufmann and M. Staemmler [Eds.], *Lehrbuch der speziellen pathologischen Anatomie*, Vol. 2. Berlin: Walter De Gruyter, 1959–1960.)

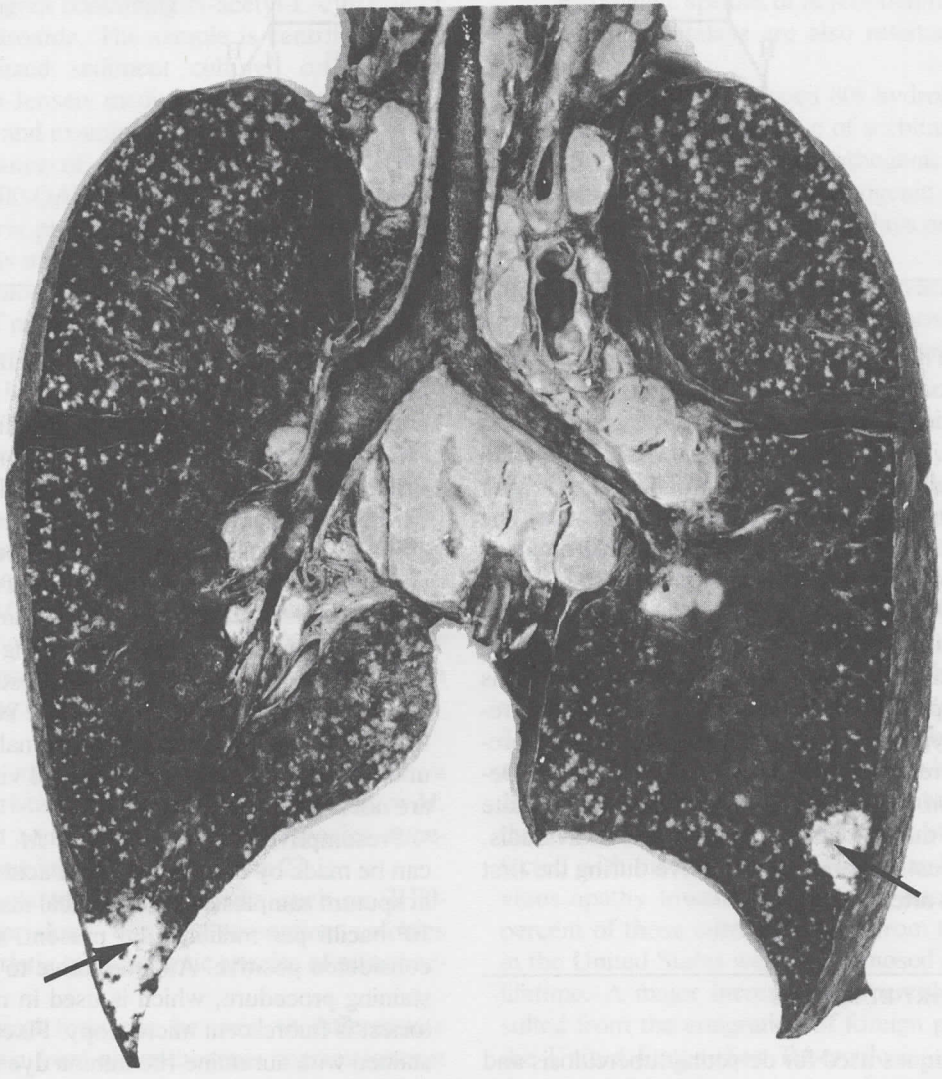
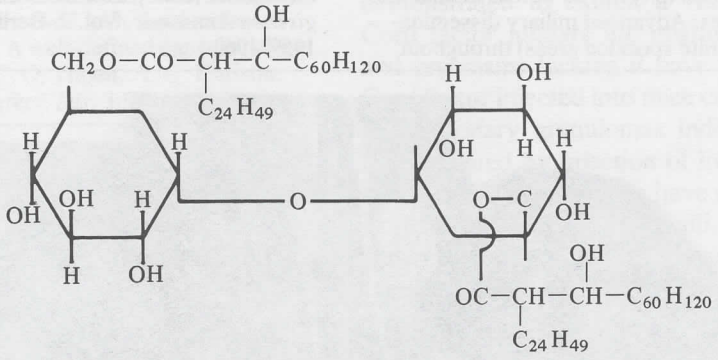


Fig. 27-4. Structure of the cord factor.



tuberculin units from 1 to 100. The standard dose in testing procedures is 5 tuberculin units. The degree of induration at the site of injection is an indication of the individual's present or past association with the TB bacillus. An induration of 1 to 5 mm after 48–72 hours is considered a negative response. The test is usually repeated, with a greater dose strength of PPD, for confirmation. If both tests are negative, the individual has presumably not had contact with the TB bacillus. An induration of 5 to 9 mm is called a *doubtful reaction*, and the test is usually repeated. An induration of 10 to 33 mm reflects a hypersensitivity resulting from either previous or present infection with the TB bacillus. Depending on their clinical presentation appropriate treatment may be necessary for these individuals. The skin test does not turn positive during the first 3–7 weeks after infection.

LABORATORY DIAGNOSIS

The techniques used for detecting tuberculosis and determining possible communicability to others are the tuberculin skin test, chest x-ray, and examination of sputum smears and cultures for *M. tuberculosis*. Because tuberculosis is primarily a disease of the lungs the major source of infecting bacilli for laboratory diagnosis is the sputum or lung secretions. When sputum samples cannot be obtained, gastric contents can be collected and examined. Tuberculosis may affect other areas of the body, and clinical material can be procured from the cerebrospinal fluid, urine, bone, joints, or feces. Confirmation of *M. tuberculosis* infection is established by identification of the bacilli in a stained smear of clinical material (smears are not performed on urine or gastric aspirations), by cultivation of the microorganisms from clinical material on suitable laboratory media, and by performing biochemical and other tests. Animal virulence tests are seldom used unless the clinical specimen contains relatively few bacilli, as in cerebrospinal fluid. Even under the best of conditions animal virulence tests are not reliable.

Presumptive identification of *M. tuberculosis* can be made by demonstration of acid-fast bacteria in sputum samples or other clinical material. When 10^5 bacilli per milliliter are present, the smear is considered positive. An alternative to the acid-fast staining procedure, which is used in many laboratories, is fluorescent microscopy. Fixed smears are stained with auramine-rhodamine dye and then observed with a fluorescent microscope. By staining with fluorochrome dyes, such as auramine-rhodamine mixture, one can readily detect the bacilli in clinical material as bright yellow-green microorganisms. Although direct microscopical examination is the least sensitive of all tests, it does indicate—especially if the clinical material is sputum—

that the patient may be highly infectious.

Accurate identification of the mycobacteria is obtained by cultural methods. Sputum samples, because of their viscosity and the presence of contaminating microbial species, are digested with a mucolytic agent containing N-acetyl-L-cysteine or sodium hydroxide. The sample is centrifuged and the neutralized sediment cultured on selective Löwenstein-Jensen medium as well as selective 7Hll media and examined weekly for 4–8 weeks for the appearance of characteristic colonies. Plated on clear 7Hl0-OADC media, pathogenic species of mycobacteria produce typical corded colonies. A niacin test is usually performed to identify the isolate as *M. tuberculosis*. *M. tuberculosis* is a strong producer of niacin. Recently, however, a species of *Mycobacterium* not pathogenic for humans has been found to be niacin positive. It is therefore recommended that combinations of various tests be used to identify and differentiate tubercle bacilli from other mycobacteria. Although tuberculosis by definition is a disease caused by tubercle bacilli (*M. tuberculosis* and *M. bovis*), other species of *Mycobacteria* can cause disease in humans (see Nontuberculous Mycobacteria). It is therefore necessary to differentiate the tubercle bacilli from the other mycobacteria. Differentiation is based largely on the following characteristics and procedures:

1. Colonies may be examined for pigmentation, a characteristic not found in *M. tuberculosis* or *M. bovis* but common to many other species, especially those in group II (see Table 27-1).
2. Species plated on clear media such as 7Hl0-OADC produce corded, serpentine colonies characteristic of pathogenic species of mycobacteria.
3. Biochemical tests can be used to differentiate pathogenic from nonpathogenic species (except *M. microti*).
   a. Niacin production. Niacin production is characteristic of human tubercle bacilli and not of other species (except *M. microti*).
   b. Nitrate reduction. Most pathogenic species can reduce nitrate, while nonpathogens do not.
   c. Catalase production. Cultures of *M. tuberculosis* heated to 68°C for 20 minutes cannot produce catalase. The nonpathogens are not affected by temperature and continue to produce catalase. It has been observed that pathogenic species of *Mycobacteria* unable to produce catalase are also resistant to isoniazid.
   d. Polysorbate 80 (Tween 80) hydrolysis. Polysorbate 80, a derivative of sorbitan monooleate, is hydrolyzed by pathogenic species in 10–20 days, while nonpathogenic species hydrolyze polysorbate 80 in 5 days or less.

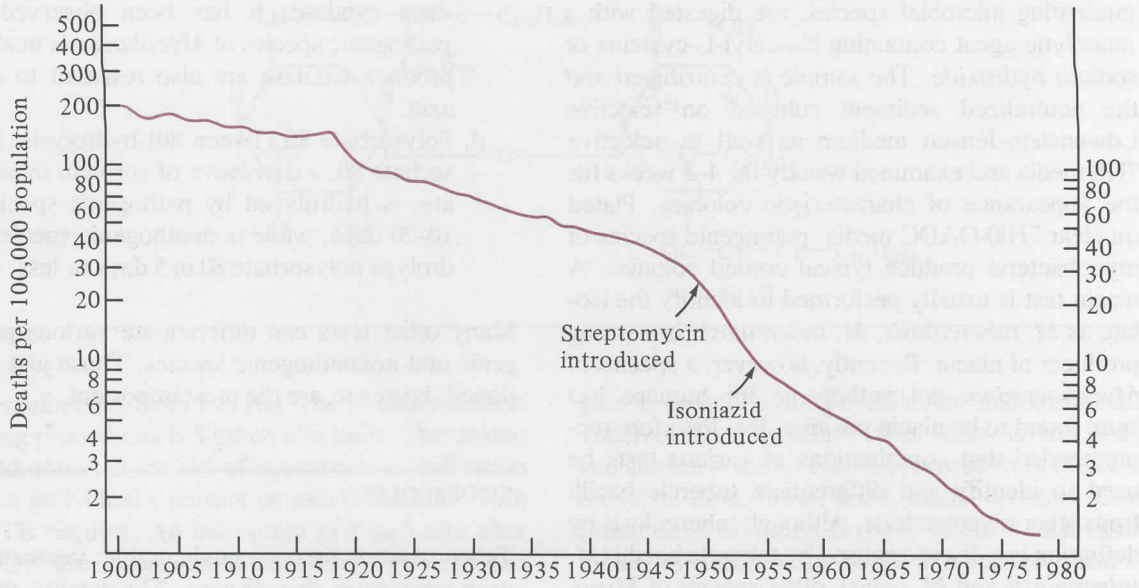
Many other tests can differentiate various pathogenic and nonpathogenic species. Those just mentioned, however, are the most important.

EPIDEMIOLOGY

Tuberculosis is treatable and curable, yet many die each year from this disease. The fatality rate in 1900 in the United States, before the introduction of antimicrobials, was approximately 200 per 100,000 population. The current rate is approximately 1.5 per 100,000 (Fig. 27-5). Tuberculosis is still a major problem in the United States, where 24,000 new cases are recognized each year. In some of the larger cities death rates as high as 6 per 100,000 are still observed. It is estimated that more than 10 million people in the United States are infected with tubercle bacilli. There has been an obvious apathy towards the disease since nearly 50 percent of those currently dying from tuberculosis in the United States were undiagnosed during their lifetime. A major increase in tuberculosis has resulted from the emigration of foreign persons into the United States (See Tuberculosis—California). Greater vigilance is required on the part of the medical community as well as the general public.

Susceptibility to tuberculosis is influenced by age. The very young (less than 5 years of age) are extremely susceptible, and in this group the disease is often fatal. Susceptibility decreases up to the early teens, then increases again, reaching a peak

Fig. 27-5. Tuberculosis death rate in the United States, 1900–1980. (From Centers for Disease Control, DHEW Publication No. 78-8322, Atlanta, GA.)



in old age. The variation in age susceptibility is associated with the effectiveness of the cell-mediated response, that is, the cell-mediated response is greatly reduced in those at either end of the age spectrum. Such factors as poor nutrition and overcrowded living conditions also play an important role in susceptibility to infection.

TREATMENT AND PREVENTION

Several drugs have been used successfully in the treatment of TB: isoniazid (INH), streptomycin, pyrazinamide, para-aminosalicylic acid (PAS), ethambutol, and rifampin. The chronic nature of the disease requires that therapy be continued for long periods of time, thus providing the opportunity for drug-resistant bacterial strains to emerge. To prevent the emergence of resistant forms of the bacilli, drugs are used in combination, for example, isoniazid plus PAS plus streptomycin, or isoniazid plus ethambutol, or isoniazid plus ethambutol plus rifampin. Until the introduction of rifampin in the late 1960s most tuberculosis chemotherapy required 18–24 months. This long-term chemotherapy revolved around the use of drugs such as isoniazid and ethambutol initially supplemented with streptomycin. Ethambutol was used to prevent the emergence of resistance to isoniazid. The discovery that isoniazid and rifampin in combination resulted in complementary bactericidal activity has permitted the length of therapy to be reduced. A (relatively) short-term chemotherapy regimen of 6–9 months can effect a cure without relapse. The short-course regimen consists of the administration of four bactericidal drugs—streptomycin or ethambutol plus isoniazid and rifampin for 6 months, followed by a 3-month regimen of isoniazid and rifampin. (Prolonged treatment with streptomycin can lead to hearing loss; therefore hearing tests should be performed.) For newly diagnosed patients with tuberculosis the combination of isoniazid and ri-

fampin may be used if the risk of isoniazid resistance is small. The drugs used in short-course therapy have potential hepatotoxic effects, but the risk is apparently small. Hepatotoxicity is observed more frequently in elderly patients. From 1 to 3 percent of the isolated pathogenic bacilli are resistant to at least one of the prescribed drugs. Mycobacterial species isolated from the patient should always be tested for antibiotic sensitivity.

Modern treatment of tuberculosis can take place in the home, and most patients do not require hospitalization. Hospitalization may be required only if the patient has an additional severe disease or if a diagnostic procedure such as lung biopsy is required. Once the patient is undergoing treatment any tubercle bacilli that might have been transmitted by the sputum are destroyed and thus transmission to healthy persons is no longer considered a factor in prevention of disease.

A person with active tuberculosis can prevent the spread of bacilli by covering the mouth and nose when coughing or sneezing. A cough may produce up to 10^5 droplet nuclei, and each nucleus may contain one or more bacilli. More than half of the droplet nuclei remain suspended in the air for at least 30 minutes. Adequate ventilation helps to prevent dissemination of the bacilli. Chemotherapy is the best method of control. Isoniazid can be used in preventive therapy as well as for treating infectious cases of tuberculosis. It is recommended for individuals who have an induration greater than 5 mm in the tuberculin skin test. Included among positive reactors who should receive chemoprophylaxis are persons with progressive tuberculosis, those who have converted recently from a negative to a positive tuberculin test, and members of households with patients with recently diagnosed tuberculosis. Isoniazid prophylaxis is usually carried out for 1 year. Patients should be carefully monitored because prolonged use of isoniazid has been known to cause liver damage. Isoniazid should be discontinued, of course, when such toxic effects can be demonstrated.

In the past patients cured by chemotherapy were advised to have follow-up examinations and x-ray tests. Today persons who have responded to treatment and are considered cured are no longer advised to return for examination. If the discharged patient develops tubercular symptoms, such as persistent fever and cough and loss of weight, a follow-up examination and x-ray should be initiated.

The bacillus of Calmette and Guérin (BCG), isolated from a strain of *M. bovis*, has been used since 1921 in a live vaccine for the prevention of TB. The vaccine has been used more frequently in other countries than in the United States. The vaccine is not recommended in the United States because it results in the recipient's having a positive tuberculin test; hence a valuable diagnostic aid is lost. Where it is used the vaccine should be administered only to individuals who are tuberculin test negative (that is, negative to 5 tuberculin units of tuberculin PPD) and who are frequently exposed to others with TB. The vaccine can also be administered to groups or in communities where new infections keep increasing even after programs of treatment have been initiated. Those receiving the BCG vaccine should have a repeat tuberculin test 3 months later. If the skin test is negative, the vaccination should be repeated. For newborns, the dose of vaccine is one-half that used for adults. If revaccination is necessary, a full dose of vaccine is administered. The vaccine should not be given to anyone who is tuberculin positive or to individuals with impaired immune responses. It has been known to cause death in children with immunological disorders. In the United States isoniazid chemoprophylaxis rather than BCG vaccine is recommended for prevention. In developed countries where the level of resistance to tuberculosis is relatively high it is much cheaper to treat the disease with drugs than to use preventive methods on a large segment of the population.

NONTUBERCULOUS MYCOBACTERIOSES

Many mycobacteria are saprophytic, while others have been associated with diseases in a variety of animals such as birds, fish, and nonhuman mammals. Some of these mycobacterial species have

TUBERCULOSIS—CALIFORNIA

California recorded 4520 new tuberculosis cases in 1981, for a rate of 18.7 per 100,000 population, compared with a national rate of 11.9 per 100,000. Since special tuberculosis hospitals and sanatoria no longer exist, tuberculosis may now be seen by any health care provider or clinic. Following is one example in which the disease was not initially considered.

On November 19, 1982, a 39-year-old Chinese-speaking housewife was discovered to have laboratory-confirmed (sputum- and culture-positive) pulmonary tuberculosis. She had immigrated from Burma in 1972, and, at that time, her chest x-ray was normal. Throughout the summer of 1982, she felt unwell and had fever and some weight loss. She sought medical advice in July, but no diagnosis was established. Her symptoms persisted, and she presented again in September with an 8-pound weight loss, a frequent cough, and fatigue. Again no diagnosis was established, and she was given vitamins. In early November, her cough became productive of heavy sputum. She returned to her physician later that month after a 2-year-old child she was tending was found on routine examination to have a positive tuberculin skin test. This child was subsequently admitted to a hospital with pleurisy and x-ray findings compatible with tuberculosis; therapy was started.

Investigation revealed that the woman tended four or five children each day. Three other children in this group (ages 2½ years, 3 years, and 21 months) were found to have positive skin test reactions and x-ray findings compatible with pulmonary tuberculosis. All three are being treated as patients. One other child also under her care had a positive reaction to purified protein derivative (PPD); treatment with isoniazid (INH) was begun. In addition, her own children (ages 5, 11, and 15 years) and her husband had positive reactions to PPD and were given isoniazid.

Refugees from Southeast Asia and other immigrants from Asia, Latin America, and the Pacific Basin are coming to California in large numbers. This trend can be expected to continue. Tuberculosis must be considered in these patients who come from high-incidence areas and present with cough and fever. Although the risk of developing current tuberculosis is greatest during the first few years after arrival, these persons remain at higher risk of developing current tuberculosis than the general population.

From *MMWR* 32:58, 1983.

Table 27-1. Characteristics of the Most Frequently Isolated Nontuberculous *Mycobacterium* Species

Runyon classification group	Species	Source of species	Clinical manifestation	Other characteristics
I	*M. kansasii*	Milk	Pulmonary disease	Pigment produced when exposed to light (photochromogen)
	M. marinum	Water	Cutaneous disease	
	M. simiae	Water (?)	Pulmonary disease	
II	*M. scrofulaceum*	Water, soil	Cervical adenitis in children	Pigment produced in dark (scotochromogen); strong resistance to many antimicrobials
	M. szulgai	Soil	Pulmonary disease, cervical adenitis	
	M. xenopi	Soil	?	
III	*M. avium*	Soil	Pulmonary disease	Nonpigmented (nonchromogens)
	M. intracellulare			
IV	*M. fortuitum*	Water, soil	Cutaneous and ocular disease	Rapid growth (3–5 days); resistance to many antimicrobials
	M. chelonae		Pulmonary disease, abscesses, postoperative wound infections, ocular disease	

been found in patients with tuberculosis-like symptoms, and in 1959 Runyon classified these species as "atypical" on the basis of their colonial morphology. The term *atypical* is no longer considered acceptable, and the diseases they cause are now known as *nontuberculous mycobacterioses*. Major characteristics of this group are given in Table 27-1.

PATHOGENESIS

Because the human pathogenic nontuberculous mycobacteria are not infectious for animals, much concerning the transmission and host response to infection remains unknown. *M. kansasii* is the most frequently isolated species from human infections. *M. kansasii, M. avium*, and *M. intracellulare* produce pulmonary infection that is indistinguishable from that produced by *M. tuberculosis* or *M. bovis*. Infection is more common in patients compromised by chronic pulmonary conditions such as emphysema and bronchitis. In all nontuberculous mycobacterioses, dissemination from the initial site of infection is rare except in some immunologically compromised patients.

A more careful examination of natural waters and community water supplies has revealed the presence of nontuberculous mycobacteria. They have often gone unrecognized because of their slow growth on laboratory media but are now recognized as common contaminants of water. They are especially troublesome in the hospital because of their resistance to disinfectants such as chlorine and glutaraldehyde, and they have been incriminated in infections following such hospital procedures as dialysis, open-heart surgery, and insertion of implant devices (for example, porcine heart valves).

LABORATORY DIAGNOSIS, TREATMENT, AND PREVENTION

Clinical characteristics may suggest nontuberculous mycobacteriosis, but repeated isolation of the organism from specimens such as sputum is required. Although *M. kansasii* is susceptible to drugs used for treatment of classic tuberculosis, the concentrations necessary for inhibition are greater than those for *M. tuberculosis*. *M. avium* and *M. intracellulare* are usually resistant not only to the drugs used in the treatment of tuberculosis but to most antimicrobials. Surgical resection of lung tissue, if possible, is suggested for successful therapy. *M. marinum* is resistant to antituberculosis drugs but susceptible to rifampin. *M. xenopi* is susceptible to antituberculosis drugs. *M. fortuitum* and *M. scrofulaceum* are resistant to most antimicrobials. There are no vaccines for immunization.

MYCOBACTERIUM LEPRAE

The causal agent of leprosy (Hansen's disease), *M. leprae*, has the potential for causing disfigurement. Infected individuals who show maximum resistance to the bacilli demonstrate a disease affecting the superficial nerve endings and related skin areas. In persons with minimal resistance the bacilli may be disseminated throughout the body.

GENERAL CHARACTERISTICS

M. leprae is an acid-fast intracellular parasite that causes infection in humans. The bacilli are usually transmitted from person to person via nasal secretions and enter the host through abrasions on the skin. The bacilli have never been cultivated on artificial media or tissue culture. Some success has been obtained by culturing the bacilli in the mouse foot pad. The nine-banded armadillo is also susceptible to leprosy. Most cases in the United States occur in California, Texas, and Hawaii.

PATHOGENESIS

Leprosy, which invariably involves the nervous system and contiguous skin areas, has an incubation period of 3–5 years. In *tuberculoid leprosy* (*TL*) lesions may appear on the skin or any part of the body but predominate on the face and extremities. The lesions on the skin are large, granulomatous, blanched, and very flat. The host exhibits more resistance in the tuberculoid form; therefore this form of the disease is usually benign. For individuals less resistant to the bacilli a more disfiguring form of leprosy called *lepromatous leprosy* (*LL*) may exist (Fig. 27-6). In LL, bacteremia is often continuous and is related to the number of bacilli in the skin. As many as 10^5 bacilli per milliliter of blood can be present in the infected patient. Patients with LL may also have erythema nodosum leprosum, an immunological complication characterized by fever and many tender red skin lesions. LL frequently progresses to the stage at which the patient becomes disfigured or blind. The long incubation period of leprosy indicates the tolerance of the host to the bacillus. The immunologic unresponsiveness of patients to *M. leprae* is believed to be due to a suppression mechanism, that is helper T cells and suppressor T cells are in some way affected by antigens of *M. leprae*. Leprosy patients lack an effective cell-mediated immune response and macrophages are unable to kill the leprosy bacillus.

LABORATORY DIAGNOSIS, TREATMENT, AND PREVENTION

Scrapings from skin, nasal secretions, or lymph fluids removed from punctured skin nodules are stained for the presence of acid-fast bacilli, which often appear in parallel rows. Infection of the mouse foot pad is sometimes used to confirm the diagnosis of leprosy. The appearance of characteristic lesions is a confirmation of the leprosy bacillus.

Dapsone, a bacteriostatic sulfone drug, has been used successfully in the treatment of leprosy. Elim-

Fig. 27-6. Lepromatous leprosy. A. Hawaiian girl 11 years of age with early lepromatous leprosy. Inconspicuous lesions are seen on cheeks and chin. B. Same girl at 13 years of age. Observe nodular thickening of right ear, lesions of cheeks and chin, and dramatic change in facial appearance. C. Forearms and hands of patient at 11 years of age. No apparent lesions. D. Hands of patient at 13 years of age. Nodular lesions are apparent on forearms and fingers. (From C. H. Binford. In W. A. D. Anderson and J. M. Kissane [Eds.], *Pathology*, Vol 1. St. Louis: Mosby, 1977.)

ination of the bacilli from infected patients often takes 3–5 months. Antimicrobial treatment is frequently continued for several years to prevent relapses. Recently a number of *M. leprae* isolates have shown a resistance to sulfones. Rifampin is considered an alternative drug for therapy. Although the infectivity of the disease is low, individuals with untreated leprosy should be isolated, particularly from young children. Adults who are being effectively treated do not have to be separated from their children. Prophylaxis is considered for family members. In some islands of the South Pacific BCG vaccine has been used with some success as a prophylactic against leprosy in children. Trials in other areas have not, however, been so successful.

SUMMARY

The *Mycobacterium* species of major clinical importance are *M. tuberculosis* and *M. bovis*, the causal agents of tuberculosis, and *M. leprae*, the causal agent of leprosy. Mycobacteria are aerobic bacilli whose growth rate is among the slowest of all bacteria. They are not stained with the usual aniline dyes, such as Gram stain dyes, because of high lipid content in the cell envelope. They are best stained by employing acid-fast staining techniques.

Tuberculosis is primarily a respiratory disease, but any organ of the body can be infected. The causal agent of disease is a facultative intracellular parasite that multiplies at the site of infection. Manifestations of disease are slow to develop, and chronic disease is the norm. Bacilli can remain inactive in lung tissue for a period of time but can later be activated to cause disease. Tuberculosis is diagnosed by identification of bacilli in sputum or other clinical specimens, cultivation of the organisms on special laboratory media, and biochemical testing. Because of the intracellular habitat of the tubercle bacilli, chemotherapy is required for a long period of time. Treatment requires the use of combination of drugs to prevent emergence of resistant strains. Isoniazid, streptomycin, ethambutol, and rifampin are some of the drugs used in the treatment of tuberculosis. Several nontuberculous mycobacteria are associated with pulmonary as well as extrapulmonary disease. They are important because of their resistance to drugs that are used in treatment of tuberculosis.

Leprosy is an infrequently observed disease whose incubation period may be several years. The disease may be manifested clinically in two forms. The lesions of one form are superficial, while the other may result in disfigurement. The causal agent of disease, *M. leprae*, cannot be cultivated on laboratory media or most animals. It can be cultivated in the armadillo. Diagnosis of disease is made by acid-fast staining of clinical specimens. Dapsone is the drug of choice in treatment.

QUESTIONS FOR STUDY

1. Why are the mycobacteria difficult to stain with aniline dyes such as those used in the Gram stain?
2. Tuberculosis is primarily a respiratory disease. How does the organism become disseminated to other organs?
3. Describe the pathological events that occur during mycobacterial multiplication in respiratory tissue.
4. What is the purpose of the tuberculin test? How is it interpreted?
5. How is tuberculosis diagnosed in the clinical laboratory?
6. What biochemical tests are used in the identification of mycobacteria?
7. List the chemotherapeutic agents used in the treatment of tuberculosis. Which one is also used prophylactically?
8. Which species of *Mycobacterium* is most frequently isolated from nontuberculous disease?
9. What is the distinction between tuberculoid and lepromatous leprosy?
10. How does the treatment for Hansen's disease differ from that for tuberculosis?

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28. SPIROCHETES AND CURVED RODS

OBJECTIVES

To outline the various symptoms associated with the stages of syphilis

To outline the types of treatment for the different groups of individuals who have syphilis

To briefly describe the most important laboratory tests used to identify the syphilis organism

To list the causative agent, method of transmission, site of infection, and method of treatment for leptospirosis, relapsing fever, rat bite fever, and *Campylobacter* infection

Spirochaetaceae (the spirochetes)
Genus *Treponema* (*Treponema pallidum*)
General Characteristics of *Treponema pallidum*
Pathogenesis
Primary Syphilis
Secondary Syphilis
Latent Syphilis
Tertiary or Late Syphilis
Congenital Syphilis
Epidemiology
Laboratory Diagnosis
Dark-Field Examination
Immunofluorescence
Serological Tests
Biological False-Positives
Chemotherapy
Prevention and Control
Nonvenereal Syphilis
Genus *Borrelia*
General Characteristics
Pathogenesis and Epidemiology
Laboratory Diagnosis
Treatment and Prevention
Genus *Leptospira*
General Characteristics
Pathogenesis and Epidemiology
Laboratory Diagnosis and Treatment
Spirochetes and Lyme Disease
Spirillaceae
Spirillum minor
Campylobacter fetus

A number of curved bacteria are indigenous to soil and water, and some can be found as commensals in animals. Only a few, however, are human pathogens. They belong to two families—the Spirillaceae and Spirochaetaceae. The important genera in the Spirillaceae are *Spirillum* and *Campylobacter*; the genera *Treponema, Borellia* and *Leptospira* belong to the family Spirochaetaceae or, as they are more familiarly known, the *spirochetes*.

SPIROCHAETACEAE (THE SPIROCHETES)

The spirochetes are long, slender, helically shaped cells that are actively motile by means of flagella-like filaments called *axial filaments*. The axial filaments are located between an outer envelope or sheath and an inner protoplasmic cylinder (see Fig. 4-22). The number of axial filaments varies depending on the genus.

Spirochetes are gram-negative (but the Gram procedure is rarely used to identify them). They are from 3 to 500 μm in length and from 0.2 to 0.75 μm in diameter. They all divide by transverse fission and may be aerobic, facultatively anaerobic, or anaerobic. Routine staining procedures are not ordinarily employed for most genera; they are best recognized by dark-field microscopy. The only reliable method for differentiation among the pathogenic spirochetes is based on the size, number, and spacing of the helical coils (Fig. 28-1).

GENUS *TREPONEMA* (*TREPONEMA PALLIDUM*)

Treponemal species can be found as commensals in the oral cavity, gastrointestinal tract, and urogenital tract of animals. The pathogenic treponemes are *T. pallidum*, the causative agent of venereal syphilis; *T. pertenue*, the agent of *yaws*, which is a tropical disease; and *T. carateum*, the agent of a chronic skin disease found in Central and South America called *pinta*. These three diseases are called *treponematoses*.

General Characteristics of Treponema pallidum

T. pallidum, the agent of venereal syphilis, is 6 to 14 μm in length with a width (0.2 μm) just ap-

Fig. 28-1. Relative size and shape of the spirochetes. A. Genus *Treponema*. B. Genus *Leptospira*. C. Genus *Borrelia*.

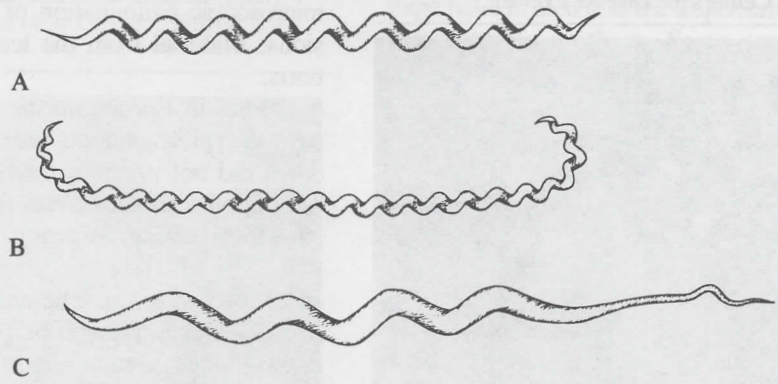
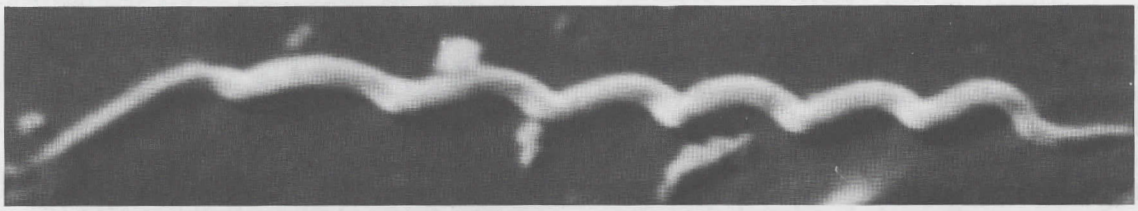


Fig. 28-2. Electron micrograph of *Treponema pallidum* attached to rabbit testes. (From T. J. Fitzgerald, *J. Bacteriol.* 130:1333, 1977.)



proaching the resolving power of the light microscope. The organism cannot be stained easily by ordinary dyes and is best observed by dark-field microscopy. When observed microscopically, the coils are seen to be evenly spaced 1 μm from each other (Fig. 28-2).

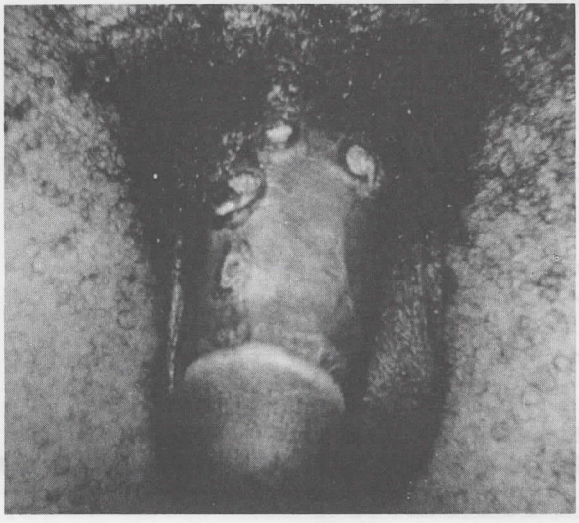
Pathogenic treponemes are not cultivable with any consistency in artificial laboratory media. They are ordinarily cultivated in rabbit testicular tissue, which provides a source of substantial numbers of treponemes. Although considered anaerobes, *T. pallidum* outside the host exhibits metabolic activities that occur only in the presence of oxygen. A functional terminal oxidase has been discovered in *T. pallidum*; thus oxygen must play some role in the metabolism of this organism during infection of the host. *T. pallidum* is sensitive to drying and temperatures above 42°C. It is extremely sensitive to heavy metals such as mercury, arsenic, antimony, and bismuth. Until the advent of penicillin in the 1940s, heavy metals such as arsenic had been used almost exclusively in the treatment of syphilis.

Pathogenesis

T. pallidum is very sensitive to normal environmental conditions outside the body. Syphilis, therefore, is almost always contracted by direct transmission. Direct contact takes place by sexual means or by accidental contact with infectious lesions such as those that occur in the mouth. Congenital transfer between mother and fetus may also occur. Almost 90 percent of the cases of syphilis are caused by sexual contact.

Untreated, syphilis either progresses through a number of stages to debilitation and death or ter-

Fig. 28-3. Primary stage of syphilis. Chancres on the penis. (Courtesy Centers for Disease Control.)



minates spontaneously with no apparent detrimental effects. The symptoms in each stage, if present, are varied and often unpredictable.

PRIMARY SYPHILIS. In more than 90 percent of the cases a single lesion appears on the cervix of the female or the penis of the male (Fig. 28-3). The primary lesion may also be evident on any cutaneous or mucous membrane surface, as on the scrotum, labia, nipples, rectum, eyelids, fingers, and mouth. At the initial site of infection, a papule, the base of which is hard but painless, develops into an ulcerated sore. This lesion is called a *hard chancre*. The time required for appearance of the chancre varies between 10 and 90 days, the average being about 3 weeks. In women a lesion on the cervix may go unnoticed. If treated immediately with antibiotics, the chancre will disappear within a week. Untreated, it may disappear spontaneously within 4–12 weeks. Accompanying the primary chancre is the development of enlarged lymph nodes near the initial lesion. Once into the lymph, the organism is capable of reaching the bloodstream and infecting other tissues.

As many as 25 percent of patients with primary syphilis have a negative serological test. At this stage the only absolute diagnostic test is dark-field microscopic examination of material from the lesions. Material from the lesions are highly infectious.

Studies in Europe during the early 1900s of untreated syphilis patients revealed that 75 percent of cases did not progress beyond the primary stage. This implies that a certain degree of immunity results from primary infection.

SECONDARY SYPHILIS. The chancre produced in the primary stage may still be present during the secondary stage. Symptoms of the secondary stage, which is the result of systemic dispersal of the organism, may appear from 6 weeks to several months after infection. They are manifested by the development of cutaneous as well as mucous membrane lesions.

In more than 80 percent of persons infected, a macular skin rash appears on the trunk or limbs but seldom on the face. The macular lesions are round and flat and fade within a few weeks. A papular rash may occur in which the lesion is raised and indurated (Fig. 28-4). The papules usually develop on the trunk and limbs as well as the palms of the hands and soles of the feet. They also appear on the face. The papular lesion may break down, resulting in ulceration. Lesions on the scalp can cause temporary loss of hair (alopecia).

Mucous membrane lesions can occur at the mucocutaneous junctions. Sometimes these lesions coalesce to form large masses called *condylomata lata* (Fig. 28-5). They are more prevalent around the anus and labia. Lesions of the mucous membrane are also common in the mouth and on tongue and tonsils. Such a lesion is known as a *mucous patch* and is raised and covered with a gray-white membrane.

The spirochete can be demonstrated in any of the cutaneous or mucous membrane lesions by dark-field microscopy. Serological examination of patients during this stage reveals high antibody titers that remain relatively high for up to 2 years after infection—after that the serological tests are often negative. Patients in the primary and secondary

Fig. 28-4. Secondary cutaneous syphilis. (From A. H. Rudolph. In P. F. Wehrle, and F. H. Top, Sr. [Eds.], *Communicable and Infectious Diseases* [8th ed.]. St. Louis: Mosby, 1976.)

Fig. 28-5. Condylomata lata during secondary stage of syphilis. (From P. S. Friedman, *Br. J. Vener. Dis.* 53:276, 1977.)

stages are highly infectious. The infectious stage usually ends when the lesions of the secondary stage have healed and the treponemes are no longer demonstrable in them. Some treponemes, however, remain viable after the disappearance of the secondary symptoms and give rise to a quiescent period called latent syphilis.

LATENT SYPHILIS. In latent syphilis a relatively high serological titer is evident, but clinical symptoms are lacking. This stage is subdivided into early and late latent periods. Early latent syphilis is a latent infection of 2 years or less. During this period the patient may suffer relapses in which secondary lesions reappear and render the patient potentially infectious. The late latent period, considered a noninfectious stage, is a latent infection of more than 2 years. This stage may last the lifetime of the patient or until certain clinical symptoms are manifested that occur in the tertiary stage of syphilis.

TERTIARY OR LATE SYPHILIS. Lesions of the tertiary stage may occur any time after the appearance of the primary and secondary stages, or they may never arise. In this noninfectious stage the lesions may or may not be debilitating. *Gummas* are the most typical tertiary lesions. Gummas contain no treponemes but rather are due to a hypersensitivity on the part of the host. They are granulomatous lesions of the skin, subcutaneous tissue, mucous membrane, bone, or viscera. Gummas of the skin (Fig. 28-6) are painless, firm, indurated nodules and may show scaling. Gummas of the subcutaneous tissue result in ulceration, with the lesion giving the appearance of being punched out. They may be found on the leg, hand, scalp, or face. Gummas of mucous membranes can eventually destroy deeper tissue such as bone and cartilage. Gummas of the mouth can lead to perforation of the hard palate as well as deformities of the soft palate and uvula. Glossitis, a superficial inflammation of the tongue, causes an initial swelling that may later result in the formation of deep fissures.

Syphilis of the bone may result in periostitis, in which there is proliferation of the bone under the periosteum, or osteitis, in which destructive lesions

Fig. 28-6. Gumma of the hand characteristic of tertiary syphilis. (Courtesy Centers for Disease Control.)

Fig. 28-7. Syphilitic aneurysm of the thoracic aorta. (Courtesy Armed Forces Institute of Pathology, AFIP 82-6064.)

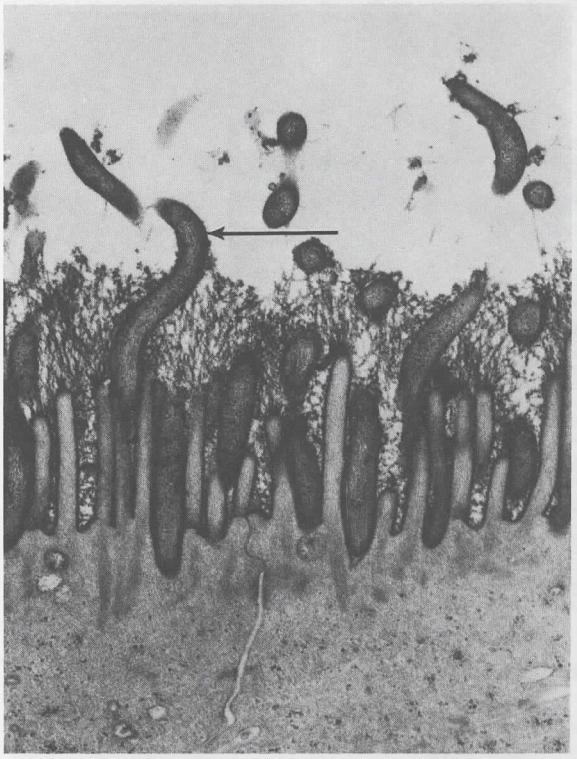
are produced. Periostitis of the tibia causes a bowing of the front of the bone and is called *saber shin syndrome*. In osteitis, flat bones, particularly those of the skull, as well as the nasal bones and the palate may be severely affected and become deformed. Gummas of the viscera are manifested most frequently in lesions in the liver.

The most destructive and debilitating lesions involve the cardiovascular and central nervous systems. The prognosis for cardiovascular syphilis is death, often as a result of aneurysms in the vessels of the heart a few months after the appearance of the symptoms (Fig. 28-7). Organisms that infect the central nervous system give rise to the following conditions: (1) In *tabes dorsalis* the spinal cord is affected, resulting in loss of control of the lower extremities; in such cases death is often the result of some other complication. (2) In *paresis*, also called *general paralysis of the insane*, there is first a personality change and then the patient becomes demented and bedridden.

Dark-field examination of tertiary lesions for spirochetes is usually negative because of the relatively few treponemes present. Serological tests can disclose the presence of antibodies in more than 70 percent of the cases. In other cases antibodies cannot be detected.

Studies on mechanisms of pathogenesis for *T. pallidum* have been difficult because of problems in cultivation. Some important aspects of pathogenesis, however, have come to light in recent years. Attachment to host cells appears to be an important step in initiating disease. The host cell receptors appear to be mucopolysaccharide, for example, hyaluronic acid, which is an important component of connective tissue. The treponemal receptor appears to be a mucopolysaccharidase located at the distal end of the treponeme (Fig. 28-8).

Fig. 28-8. Electron micrograph (×20,000 before 13% reduction) of spirochetes (*arrow*) attached end-on to the luminal cells of the colon. (From K. Hovind-Hougen, et al., *J. Clin. Microbiol.* 16:1127, 1982.)



Most tissue contains some mucopolysaccharide, and preferential attachment by treponemes appears to be associated with the mucopolysaccharide content. Those tissues with high levels of mucopolysaccharide are the dermis, aorta, eye, placenta, and umbilical cord. The treponemes attach via their mucopolysaccharidase with substrate mucopolysaccharide at the surface of the host cell. As the microorganisms multiply the mucopolysaccharides of the infected tissues are believed to be broken down, allowing dissemination to adjacent tissue. It is surmised that tissue destruction reflects mucopolysaccharide content and explains the pathology of the three stages of syphilis. The inability to isolate treponemes during tertiary syphilis, however, implies that the associated lesions are the result of the hypersensitivity of the host and thus have an immunological basis.

CONGENTIAL SYPHILIS. A mother with untreated primary or secondary syphilis is a potential threat to the fetus. After the sixteenth week of pregnancy the fetus is vulnerable to infections by *T. pallidum*. Treatment of the mother before the sixteenth week almost always prevents infection in the newborn. Treatment after the sixteenth week brings about an in utero cure but may not be in time to prevent bone or joint involvement, deafness, or interstitial keratitis. If infected fetuses are left untreated, 25 percent will die as stillbirths or spontaneous abortions, another 25 percent will die soon after birth, and 50 percent of the survivors will have severe infection. Although many infected infants appear healthy at birth, after 2 weeks symptoms begin to manifest themselves. The syphilitic is often born with or develops a rash similar to that incurred in secondary syphilis. The rash is papular and may cover the entire body but is particularly evident on the palms, soles, and areas about the mouth. Mucous patches may also appear. In the nose these patches give rise to a nasal discharge called snuffles. Snuffles often progresses to inflammation of the bone (osteitis), causing a deformity referred to as *saddle nose*. The liver is often enlarged, causing a distended abdomen. The bones can also be affected, resulting in periostitis of the long bones, a very painful condition. (See boxed essay, p. 601.)

After the second year of life noninfectious manifestations of congenital syphilis occur. Gummas can destroy the nasal septum and perforate the soft and hard palates. Periostitis of the tibia leads to the bowing of the leg known as saber shin (Fig. 28-9). The most common lesion is interstitial keratitis, which can involve both eyes. Each attack leaves some scarring on the cornea that eventually impairs vision. Hutchinson's teeth, a condition in which the central incisors of the permanent teeth are notched and translucent, is also a common late congenital symptom. Failure of the maxilla to develop makes the jaw look prominent, producing a bulldog appearance.

Fig. 28-9. Saber shin characteristic of congenital syphilis. (Courtesy Centers for Disease Control.)

Epidemiology

From 1969–1975 there was a slow but gradual increase in the number of reported cases of primary and secondary syphilis, but the data from 1977 indicated a moderate leveling off. A gradual increase has since been observed (Fig. 28-10). The number of latent and late syphilis cases, moreover, has dropped precipitously, indicating that more of the primary and secondary cases are receiving treatment. Unfortunately, many cases of syphilis in all stages go unreported and often undetected. It has been estimated that the United States has nearly 400,000 cases of all stages of syphilis.

Of the total number of reported cases of primary and secondary syphilis, more than 45 percent occur in those 15–24 years old. In 1972 a great effort on the part of state and local governments was made in the area of venereal disease education and control. It included the establishment of neighborhood health centers where screening, treatment, and follow-up procedures were available. Hopefully, these efforts will eventually lead to a decrease in primary and secondary cases of syphilis.

The total number of cases of congenital syphilis dropped considerably between 1962 and 1979: approximately 4000 cases in 1962 as compared to 357 in 1979. Evidently better testing measures and medical care are being provided in the treatment of women during pregnancy.

Laboratory Diagnosis

DARK-FIELD EXAMINATION. Examination of exudate from syphilitic lesions or rashes by dark-field microscopy is the only absolute diagnostic test for *T. pallidum*. Such examination cannot be used in all stages of syphilis because suspected lesions may not exist or may be inaccessible. The suspected lesions may also reveal no spirochetes if they are from the tertiary stage of syphilis or if the patient has received antibiotic treatment before examination.

IMMUNOFLUORESCENCE. The exudate from tissue can be fixed to a slide and stained with a fluorescein-labeled antitreponeme serum. Identification is made by fluorescence microscopy.

CONGENITAL SYPHILIS AMONG NEWBORNS—TEXAS, 1981

Thirty-seven cases of congenital syphilis were reported among newborns in Texas during 1981. An increase of 164 percent over the 14 cases reported in 1980, this represents the highest number since 1971 when 52 cases were reported. Reported cases of early syphilis among women in Texas increased 47.3 percent from 2024 in 1980 to 2982 in 1981.

Data submitted with each congenital syphilis case were reviewed, and cases were classified. No case was classified as definite; 25 (67.6 percent) were classified as probable, based on serological evidence and clinical findings; 8 (21.6 percent) as possible, based on serological evidence alone; and 4 (10.8 percent) as unlikely. The unlikely cases for which the diagnosis appeared to be based solely on evidence of maternal infection at delivery time have been excluded from the analysis that follows.

Of 33 infants evaluated, 5 (15.2 percent) were white, not of Hispanic origin; 16 (48.5 percent) were black, not of Hispanic origin; and 12 (36.4 percent) were Hispanic. Fourteen were male and 19 female. Thirty-one were live births, and two were stillborn. Two infants died within 6 days of delivery; prematurity was listed as contributing to both deaths.

The mean age of the 33 mothers was 21.7 years. Ten (30.3 percent) were 15–19 years; none of these was married. Among all 33 mothers, seven were married, three were separated, and 23 were single. The stage of the mother's infection at delivery time was primary or secondary in eight instances (24.2 percent) and early latent (< 1 year) in 23 (69.7 percent); two patients (6.1 percent) had late syphilis.

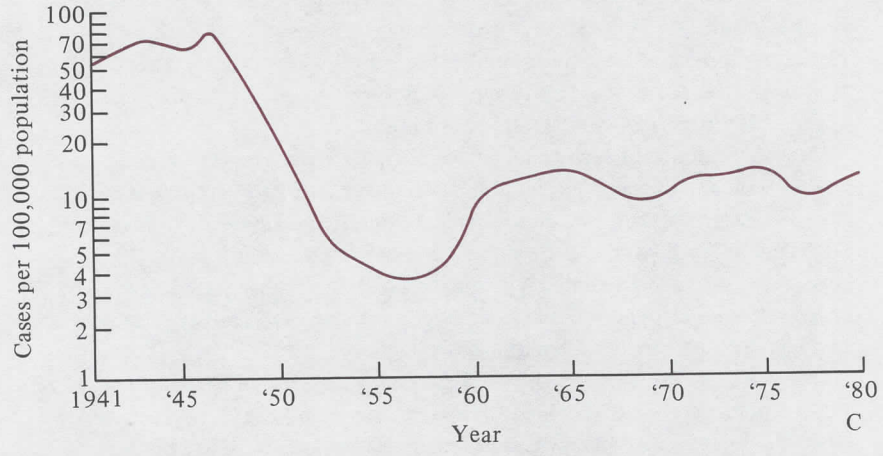
Twenty-five of the 33 mothers received no prenatal care. Of the eight who received prenatal care, five began seeing a physician late in the third trimester, and three who had negative results of serological tests for syphilis (STS) during the first trimester did not have a repeat STS in the third trimester.

Congenital syphilis is completely preventable. Its development can be related to two factors: when the pregnant woman becomes infected and how long she remains infected. Detection of untreated syphilis before delivery and prompt administration of appropriate therapy can often prevent congenital infection and can usually prevent complications among fetuses infected in utero.

Congenital syphilis can be eliminated or greatly reduced by: (1) preventing the spread of syphilis in the heterosexual community through rapid and thorough epidemiological investigations; (2) educating women at risk of infection about the need to seek early and continuous prenatal care; and (3) encouraging medical care providers to perform serological tests for syphilis on patients in the third, as well as the first, trimester.

From *MMWR* 31:382, 1982.

Fig. 28-10. Reported civilian case rates by year of primary and secondary syphilis in United States, 1941–1980. (From Centers for Disease Control.)



SEROLOGICAL TESTS. Two types of antibodies are produced in response to treponemal infections: *reagin*, a nontreponemal antibody, and a specific antitreponemal antibody. Several tests can be used to detect each. None of the tests, however, is absolute because biological false-positives can occur in any of them.

Reagin is a nonspecific antibody produced by all syphilitics, but it is not a protective antibody. It is a mixture of IgG and IgM antibodies produced in a number of other conditions and diseases besides syphilis. Reagin reacts with a cardiolipin extracted from beef heart. The most common tests used to detect the reagin-cardiolipin complex are the flocculation and complement fixation tests: the Wassermann, Kahn, Kolmer, and Hinton tests. Today they are referred to as *STS* or *serological tests for syphilis*.

1. The flocculation test is usually called the *Venereal Disease Research Laboratories* (*VDRL*) test. A positive reaction is the appearance of discrete clumps when the cardiolipin antigen is added to the patient's serum. The VDRL is employed primarily as a screening test in the laboratory. The rapid plasma reagin (RPR) card test is a modification of the VDRL test. It also detects reagin but uses charcoal particles so the test can be read macroscopically. A positive reaction is the clumping of the charcoal particles. The RPR test is used mainly in the field in surveying large groups such as migrant workers. The VDRL and RPR tests are very sensitive in the late primary and secondary stages. They are least sensitive in early primary and late latent syphilis.
2. The complement fixation test has been discontinued by many laboratories because of its lack of specificity and the exacting techniques required for its performance.

Antitreponemal antibody tests are more specific than the nontreponemal antibody tests and are used mostly to resolve problems that arise from the initial screening procedures.

1. The *T. pallidum* immobilization (TPI) test uses the live syphilis organism (extracted from infected rabbit tissue) as antigen. The patient's serum is mixed with the organisms and complement. If the serum contains specific antibody,

the organism will no longer exhibit motility and will thus become immobilized. This test is now being replaced by the fluorescent treponemal antibody test in most laboratories.

2. The fluroescent treponemal antibody (FTA) test is sensitive in all stages of syphilis. The most widely used variant of this test is the FTA absorption (FTA-ABS) test, in which nonspecific treponemal antibodies are removed from the test serum by absorption with an extract of Reiter protein (which is prepared from a nonpathogenic treponemal strain called the Reiter strain). The test is performed as follows:
   a. Killed *T. pallidum* is fixed to the slide.
   b. The patient's serum is applied to the slide and incubated for 30 minutes. If antibodies to *T. pallidum* are present in the serum, they will bind to the spirochete.
   c. Goat or rabbit anti–human globulin previously tagged with fluorescein is added to the slide. If the test is positive, a fluorescein-antibody-spirochete complex will be formed. The immunoglobulin to which the fluorescein dye is conjugated is IgG. For the detection of congenital syphilis IgG human immunoglobulin has been replaced by IgM in some laboratories. It has been found to be more specific than any of the STS tests generally used for congenital syphilis because total IgM is elevated in the umbilical cord or blood during syphilis infection.
3. *T. pallidum* hemagglutination (TPHA) tests have become popular in recent years. Red blood cells from certain animal species plus Reiter treponeme components are used. The test detects, by hemagglutination, the presence of treponemal antibodies. This test is very inexpensive and easy to perform. It compares favorably with the FTA-ABS test except in primary syphilis.

BIOLOGICAL FALSE-POSITIVES. In some individuals, serological tests for syphilis are positive when in fact the patient does not have and never had syphilis. Such tests are called *biological false-positives* (BFP). Any serological test for syphilis may give a false-positive. Reagin, which is what the VDRL test detects, can be produced in a number of conditions other than syphilis, including immunization, drug addiction, lupus erythematosus, connective tissue diseases, and hepatitis. Pregnant women, diabetics, and persons with immune disorders also sometimes produce reagin. BFP in the FTA-ABS test may result from the appearance of abnormal immunoglobulins in the patient's serum arising from any number of immune disorders. Most studies have shown that 1 in 4000 nonsyphilitic patients will give a BFP. Chronic BFP's may be a clue to certain underlying diseases or may represent latent or asymptomatic treponemal disease.

Antitreponemal antibodies persist for years after successful antibiotic therapy, while reagin declines more rapidly. A positive test for antitreponemal antibodies could also be considered a false-positive since antibodies may be present in the absence of infection.

Chemotherapy

Penicillin is the drug of choice in the treatment of all stages of syphilis. Because the generation time of *T. pallidum* is 30–33 hours, it is important to maintain a level of antibiotic to cover a sufficient number of divisions. The following are the Venereal Disease Control Advisory Committee's recommendations for the treatment of syphilis:

1. Primary, secondary, and latent syphilis of less than 1 year's duration: Penicillin G benzathine, aqueous penicillin G procaine, or penicillin G procaine in oil may be administered intramuscularly. If the patient is allergic to penicillin, tetracycline or erythromycin may be administered orally.
2. Syphilis of more than 1 year's duration (including latent syphilis, cardiovascular syphilis, and neurosyphilis): Penicillin G benzathine or aqueous penicillin procaine may be injected intramuscularly. Tetracycline or erythromycin can be used for those allergic to penicillin.
3. Syphilis in pregnancy: Penicillin is given in dosage schedules appropriate for the stage of syphilis as recommended for the treatment of non-

pregnant patients. Tetracyclines are not recommended because of their toxic effect on both patient and fetus.

4. Congenital syphilis: Aqueous penicillin G, aqueous penicillin G procaine, or penicillin G benzathine may be administered, depending on the extent of infection and condition of the cerebrospinal fluid.
5. Neurosyphilis: A total dose of 6.0–9.0 million units of penicillin G over a 3 to 4 week period is satisfactory for over 90 percent of the cases.
6. Follow-up and retreatment: All patients with early syphilis and congenital syphilis are encouraged to repeat quantitative nontreponemal tests 3, 6, and 12 months after treatment. Patients with syphilis of more than 1 year's duration should also have a repeat serological test 24 months after treatment. Retreatment should be considered when
   a. Clinical signs or symptoms of syphilis persist or recur.
   b. There is a fourfold increase in titer of a nontreponemal test.
   c. An initially high-titer nontreponemal test fails to show a fourfold decrease within a year.

In many cases of syphilis a local or generalized reaction follows the administration of penicillin. Called the *Jarisch-Herxheimer reaction*, it is characterized by headache, fever, and joint pains followed by an intensification of the syphilitic symptoms, particularly those of the secondary stage. Steroid hormones can prevent or diminish the reaction. Early investigators believed that the reaction was caused by toxic substances released from the reaction between dead or dying treponemes and sensitized syphilitic tissue. Endotoxin was thought to be the toxic substance since treponemes have a gram-negative envelope, but the endotoxin has not been detected during disease. An alternative hypothesis is that treponemal antigens released in the infected tissue form immune complexes that cause release of endogenous pyrogen from macrophages, which in turn causes fever. Herxheimer-like reactions have also been detected in other diseases including borreliosis, brucellosis, and trypanosomiasis.

Prophylactic use of penicillin before intercourse is not recommended because of the danger of masking the usual symptoms of syphilis.

Prevention and Control

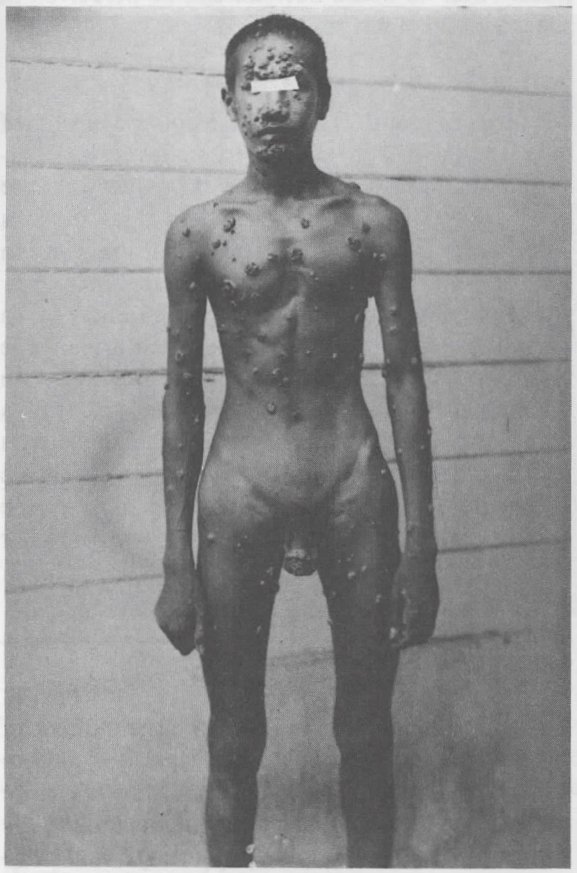
To date no active immunizing agent has been produced as a prophylaxis to syphilis infection. Although sexual abstinence would certainly be the ultimate weapon against this disease, that proposal would not be met with a great deal of enthusiasm. Control of the disease is difficult because of the high rate of travel, mildness of symptoms, infectiousness in early stages, and ignorance about the transmission, diagnosis, and treatment of syphilis. Prevention should be the ultimate goal, and this can be accomplished by

1. Education. The transmission, diagnosis, and treatment of syphilis among all age groups, especially the young, must be understood.
2. Routine serological testing programs. Many infected individuals unaware of their condition have been detected by these programs, which include testing before issuance of marriage licenses, testing of patients admitted to the hospital, and preemployment examination.
3. Investigation—as rapid as possible—concerning the treated syphilitic so that his or her contacts can be found, tested, and if necessary treated for possible infection.

Nonvenereal Syphilis

Yaws, bejel, and pinta are three diseases that resemble syphilis. They are endemic, nonvenereal, and transmitted primarily by contact with eating and drinking utensils in areas lacking adequate hygiene. Yaws is caused by *T. pertenue*, which is morphologically and serologically indistinguishable from *T. pallidum*. Yaws is endemic in Africa, India, and parts of South America. The primary lesion, called a "mother yaw," heals and later develops into a secondary lesion (Fig. 28-11). Tertiary lesions may involve the skin and occasionally the bones. Penicillin is effective in treatment.

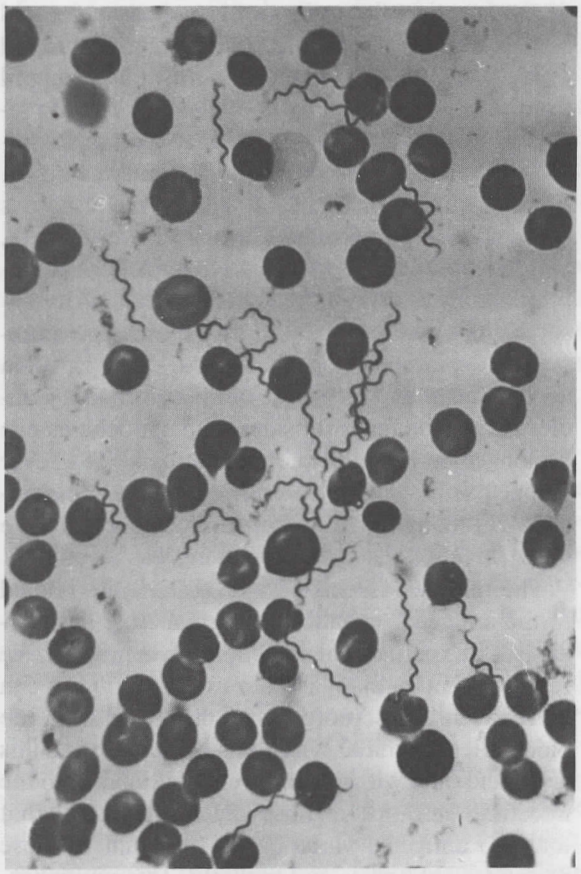
Fig. 28-11. Characteristic yaws lesions. (Courtesy Air Force Institute of Pathology, AFIP 39201.)



Pinta, a disease found in Central and South America, is caused by *T. carateum*. This organism, like *T. pertenue*, is morphologically and serologically indistinguishable from *T. pallidum*. Primary lesions develop that do not become necrotic but do become hyperpigmented. As the lesions heal the affected skin area becomes depigmented. Penicillin is effective in treatment.

Bejel is an endemic syphilis occurring primarily in the Middle East. The disease is usually contracted during childhood and is treatable with penicillin.

Fig. 28-12. *Borrelia* species (× 1000) in rodent blood. (From W. Burgdorfer. In E. H. Lennette, et al. [Eds.], *Manual of Clinical Microbiology* [3rd ed.]. Washington, DC: American Society for Microbiology, 1980.)



GENUS *BORRELIA*

General Characteristics

The borrelias are the largest of the pathogenic spirochetes, ranging in size from 10 to 30 μm in length and 0.3 to 0.7 μm in width (Fig. 28-12). They are actively motile, exhibiting a lashing, twisting movement. They have irregularly spaced coils. They are distinguished from the other spirochetes in that they can be stained by ordinary dyes such as crystal violet or carbolfuchsin. When artificial media

are supplemented with serum or blood, these organisms can be cultivated in the laboratory.

Pathogenesis and Epidemiology

The genus *Borrelia* contains species that are normally parasitic for arthropods such as ticks and lice. Humans acquire infection through arthropod bites. *B. recurrentis* is the only parasite transmitted from person to person by body lice. Tick-borne relapsing fever is caused by several species of *Borrelia*. The principal species in North America are *B. hermsii, B. parkeri*, and *B. turicatae*. The disease is more prevalent in eastern Europe than elsewhere, and few cases are reported in North America. Approximately 3–4 days after infection there is an onset of chills and fever caused by the excessive numbers of spirochetes in the blood. This is often accompanied by tenderness of the spleen and jaundice. Later the fever subsides, and spirochetes can no longer be found in the bloodstream. The fever reappears, however, 4–14 days after the initial attack. As many as 10 relapses may occur after the initial infection, each one becoming less severe.

The relapses associated with disease are related to the antigenic variation of the infecting microorganism. Experimental infections in animals have revealed that as many as four major serotypes can be recovered. Microorganisms disappear from the blood after the initial symptoms of disease, and this is coincidental with a high level of antibodies to the infecting serotype. Those microorganisms that undergo antigenic variation are resistant to these particular antibodies and multiply. This multiplication cycle results in the return of clinical symptoms. Once the immune system has responded to this new serotype, the clinical manifestations of disease disappear. Four or more relapses can occur, each shorter than the last. The duration of relapses in tick-borne disease is approximately 14 days, and only 5–9 days in louse-borne disease.

Laboratory Diagnosis

Borrelias are easily observed in stained blood smears, provided the blood is sampled during the fever period. The organisms can also be detected in the unstained preparation by phase-contrast microscopy. When spirochetes cannot be detected in blood smears, a blood sample from the infected patient can be injected intraperitoneally into white mice. After 48 hours *Borrelia* can be perceived in the tail blood of the infected mouse.

Treatment and Prevention

Tetracyclines and chloramphenicol are drugs of choice in the treatment of relapsing fever. If not effecting an immediate cure they do reduce the rate of relapses. Tetracycline treatment does incite a Jarisch-Herxheimer–like reaction, but the reaction is not as severe if penicillin is used in the treatment.

No prepared vaccines have proved effective to date. Prevention is therefore centered around the control of the insect vectors, lice and ticks. In building sites where ticks may be present, sprays containing 1 percent aldrin or 0.5 percent malathion can be applied. Persons going into areas that are potential tick habitats should cover as much of the body as possible and check the skin periodically for the presence of ticks.

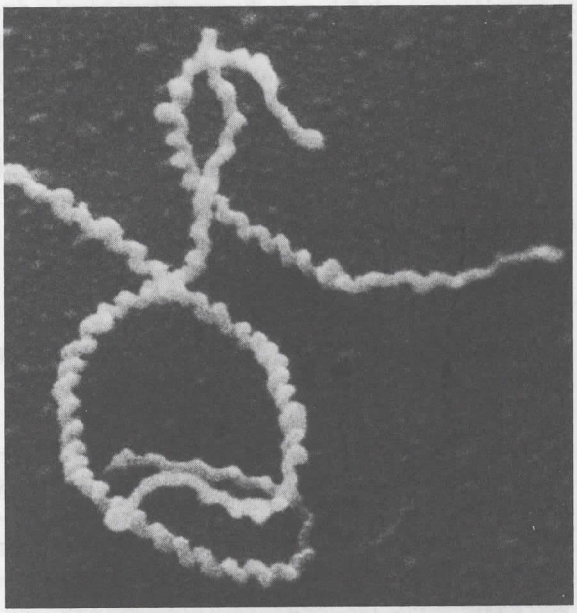
GENUS *LEPTOSPIRA*

The genus *Leptospira* contains a large number of species that are saprophytic and harmless to both humans and animals. Several species, however, are parasitic and cause infections characterized by their effect on the liver, kidneys, lungs, and meninges. The pathogenic leptospires are classified under the species name *L. interrogans*, with serogroups such as *canicola, pomona*, and *icterohaemorrhagiae*. Each serogroup can be divided into serologically distinct types called serovars.

General Characteristics

Leptospires are thin and spiral shaped, range in length from 6 to 20 μm, and are 0.1 μm in width. The spirals have numerous coils and hooked ends (Fig. 28-13). The coils can be observed when a living culture is examined by dark-field microscopy. The leptospires are not readily stained by ordinary dyes but can be perceived in stained preparations by means of the silver impregnation method.

Fig. 28-13. Scanning electron micrograph of a leptospire. Note the compactness of the coils. (Courtesy Z. Yoshii.)



Most species can be cultivated on ordinary laboratory media supplemented with animal serum or serum fractions. The organisms are obligate aerobes.

Pathogenesis and Epidemiology

The pathogenic leptospires are harbored by wild rodents and domestic animals. It has been estimated that as many as 10 to 30 percent of the wild rat population are hosts to them. *L. icterohaemorrhagiae* is the most pathogenic serogroup, although most of the other serogroups produce some disease symptoms. In wild rodents, in which the infection is almost never fatal, the organisms localize in the kidneys and are shed into the urine. Up to 1 million leptospires per milliliter can be excreted by infected rodents. Transmission from animals to humans is usually indirect. Animal urination in soil or water, for example, is a frequent mode of transmission. Swimming or other recreational activities that bring individuals, especially teenagers, young adults, and household pets, in contact with contaminated water is responsible for outbreaks of disease.

The organism multiplies in the blood and can infect other areas of the body, primarily the liver, kidney, lung, and meninges. The incubation period of 8–12 days is followed by chills, fever, headache, and severe muscular pain. The most serious form of leptospirosis, called *infectious jaundice* or *Weil's disease*, is often characterized by jaundice and hemorrhage in the skin and subcutaneous tissue (hence the serogroup name, *L. icterohaemorrhagiae*). The disease is frequently diagnosed improperly because jaundice appears in only 50 percent of infected individuals. Since there is no vaccine against the organism, prevention is achieved by avoiding contact with contaminated water.

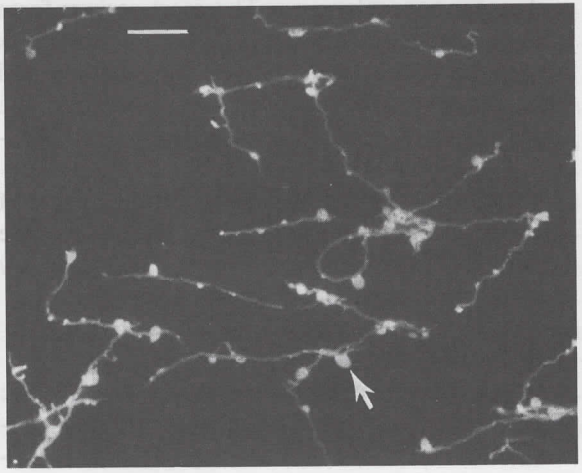
Laboratory Diagnosis and Treatment

During the first week of disease, blood and urine samples can be collected and introduced into suitable media for cultivation of leptospires. Leptospires can also be detected in the blood by dark-field microscopy. Leptospires can be detected serologically by using a variety of agglutination tests such as microscopic agglutination, indirect hemagglutination, and the enzyme-linked immunosorbent assay (ELISA). Antibodies can also be detected after 1 week of the illness by various agglutination tests. If diagnosis of leptospirosis is made during the early stages of infection, penicillin, streptomycin, or tetracycline is an effective chemotherapeutic agent. Once the disease has progressed beyond the fever stage, antibiotic therapy is ineffective.

SPIROCHETES AND LYME DISEASE

Lyme disease is a systemic illness characterized by a distinctive annular, expanding, erythematous skin lesion (called *erythema chronicum migrans* or *ECM*) and may be accompanied by acute arthritis, cranial neuritis, meningoencephalitis, or cardiac involvement. The causal agent, which has recently

Fig. 28-14. Lyme disease spirochetes. A. Indirect fluorescence of fixed spirochetes isolated from *Ixodes* ticks and incubated with monoclonal antibodies. Arrow points to outer membrane bleb. Bar = 5 μm. B. Phase-contrast micrograph after illumination with epifluorescence and low-intensity transmitted light. Bar = 5 μm. (From A. G. Barbour, S. Tessier, and W. Todd, *J. Infect. Immun.* 41:795, 1983.)



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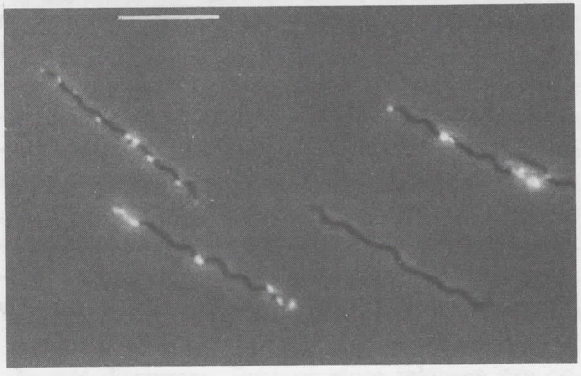

B

been classified taxonomically as *Borrelia*, is a spirochete associated with the ixodid tick. Arthritic manifestations may appear from weeks to 2 years after the erythematous lesion disappears.

A history of tick bites, outdoor activities, or pets carrying ticks is associated with most cases of Lyme disease. The Lyme disease–associated spirochete can be isolated from *Ixodes* ticks and cultivated in cell-free media. The organisms have been detected by indirect immunofluorescence (Fig. 28-14).

The diagnosis of Lyme disease is currently dependent on clinical grounds and the appearance of the ECM lesions as well as epidemiological data. Antibiotic chemotherapy can alter the course of the disease. Penicillin V or tetracycline can successfully affect the early phases of disease when ECM is present and can reduce the more severe complications of disease.

SPIRILLACEAE

The Spirillaceae are motile, gram-negative, rod-shaped and curved bacteria. Their motility arises from the presence of polar flagella and not axial filaments. Several genera are found in fresh water and seawater, but the pathogenic genera *Spirillum* and *Campylobacter* are found in animals.

SPIRILLUM MINOR

S. minor is the only human pathogen in the genus *Spirillum* and is the cause of a form of *rat-bite fever*. The organism has not been cultivated in the laboratory. Identification was originally derived from the experimental inoculation of humans with blood containing the microorganisms.

Rat-bite fever is a disease of rats that is transmissible to humans by the bite of an infected animal. The incubation period lasts 6–14 days, followed by the swelling of the wound site and formation of an ulcer. There is fever and swelling of local lymph nodes. The fever is undulating and may continue for several weeks. During the fever period a maculopapular rash appears on the arms, legs, and trunk but subsides with a drop in the patient's temperature. The disease is self-limiting unless a secondary infection arises at the site of the bite, and antibiotic therapy is not ordinarily required. Penicillin is the

drug of choice when complications do arise.

Diagnosis of disease can be made by microscopic examination of blood or material from the bite. The organisms can be stained by the Giemsa or Wright technique. If this is not possible animals must be inoculated and their blood examined for the organisms.

CAMPYLOBACTER FETUS

Campylobacter fetus is the only human pathogen in the genus *Campylobacter*. The organism is a gram-negative, microaerophilic, comma-shaped rod possessing a single unipolar or bipolar flagellum (Fig. 28-15). Two subspecies of *C. fetus* infect humans: *C. fetus* subspecies *jejuni*, which is usually associated with gastrointestinal disease, and *C. fetus* subspecies *intestinalis*, which is a cause of disease in compromised patients such as the immunosuppressed. We are primarily concerned with *C. fetus* subspecies *jejuni*.

C. fetus subspecies *jejuni* is found in many animal species and is an important agent of disease. The organism is one of the most common causes of bacterial diarrhea in the world. Epidemiological evidence has linked human disease to the consumption of contaminated food, particularly poultry, raw milk, and water as well as contact with infected animals such as goats, dogs, cats, and sheep. The organism can survive for several weeks in water. (See boxed essay, p. 610.)

Disease is characterized by diarrhea, abdominal pain, nausea, vomiting, and fever. Some patients have bloody stools, and the abdominal pain may mimic appendicitis. Most patients recover in a few days, but occasionally severe disease may be incurred. *Campylobacter* can cause infectious proctitis in homosexual men.

Diagnosis of disease can be made by inoculating fecal specimens into selective media. Plates must be incubated at 42°C for 48 hours in an atmosphere that contains 5% oxygen and 10% carbon dioxide. Colonies can be Gram-stained or examined by dark-field microscopy.

When antibiotic treatment is required (prolonged

Fig. 28-15. Electron micrograph of *Campylobacter fetus* subspecies *jejuni* (×33,740 before 37% reduction). Note curved shape of organisms and polar flagella. (From P. J. Pead, *J. Med. Microbiol.* 12:383, 1979.)

CAMPYLOBACTERIOSIS ASSOCIATED WITH RAW MILK CONSUMPTION—PENNSYLVANIA

During May 1983, two outbreaks of gastrointestinal illness after consumption of raw milk occurred in Pennsylvania. A total of 57 people became ill.

The first outbreak occurred after a visit by 60 first-grade students and three teachers to a dairy farm in south central Pennsylvania. Thirty one (49 percent) of the 63 visitors became ill, but no acute gastrointestinal illnesses were reported by members of the farm family. Symptoms included fever greater than 102°F (85 percent), abdominal pain (81 percent), vomiting (55 percent), diarrhea (52 percent), headache (13 percent), bloody stool (10 percent), and myalgia (7 percent). Onset of disease ranged from 1 to 8 days. Illness lasted from 5 hours to 12 days. Sixteen persons saw a physician; none was hospitalized. *Campylobacter jejuni* was found in the stool of the only child whose stool was cultured. Secondary illnesses compatible with *Campylobacter* infection occurred in two households.

Cookies and small cups of raw milk were served at the farm. Each of the 63 visitors drank one cup of raw milk and ate one cookie. Cultures of the raw milk from the farm did not yield *Campylobacter*. No dairy cattle were reported to have been ill, and no specimens from cattle were cultured.

The second outbreak occurred on May 20, when 45 persons (43 kindergarten children and two teachers) visited a dairy farm in central Pennsylvania. Subsequently, 26 persons (58 percent) developed gastrointestinal illness characterized by abdominal pain (73 percent), diarrhea (69 percent), fever (58 percent), nausea (54 percent), headache (50 percent), fatigue (38 percent), vomiting (19 percent), bloody stools (12 percent), and myalgia (8 percent). The incubation period ranged from 2 to 10 days. Duration of illness was 1–14 days. Four children saw a physician, and one was hospitalized. *C. jejuni* was found in two of two stool specimens cultured.

Raw milk and cookies were also served at the second farm. Illness was associated with quantity of milk consumed. Twenty-five of the 26 ill persons each consumed one-half cup or more of raw milk, and 10 of 15 well persons each consumed the same amount of raw milk. Illness was not associated with eating cookies, touching farm animals, or consuming raw milk from other sources, or with the presence of animals in the home. Members of the farm family routinely drank raw milk, and none reported illness. There were no illnesses among the herd, and no specimens from cows were cultured. Gastrointestinal illnesses, probably representing secondary transmission occurred in households of six patients.

From *MMWR* 32:337, 1983.

illness or complications), erythromycin is the drug of choice.

SUMMARY

Important spiral and curved bacteria that are human pathogens include the spirochetes (genera *Treponema, Borrelia*, and *Leptospira*) and the genera *Campylobacter* and *Spirillum*.

Spirochetes are motile by means of axial filaments that surround the cell but are not in contact with the external environment, while *Campylobacter* and *Spirillum* possess external flagella. All genera are gram-negative.

Treponema pallidum is the causal agent of a venereal disease called syphilis, while diseases called nonvenereal syphilis are caused by species that resemble *T. pallidum* (*T. pertenue* and *T. carateum*). Syphilis can be divided into four stages. The primary stage is characterized by the formation of a chancre, the secondary stage by the formation of a rash, the latent stage by the lack of symptoms, and the tertiary stage by granulomatous lesions. Most cases of syphilis are caused by sexual contact, but up to 10 percent are caused by other means. Syphilis is also a congenital disease that may result in death of the fetus or congenital lesions affecting any organ.

Syphilis can be diagnosed in the laboratory by dark-field microscopy or immunofluoresence if clinical specimens containing the spirochete are available. Serological tests are the tests most routinely used. They are based on identification of specific antibodies (*T. pallidum* immobilization test, fluorescent treponemal antibody test, and *T. pallidum* hemagglutination) or nontreponemal antibodies (Venereal Disease Research Laboratories test and rapid plasma reagin test). Serological tests may give false-positives.

Prevention of syphilis is based on education, early diagnosis of infected individuals and their contacts, and routine serological diagnosis. Penicillin is most effective in the treatment of disease.

Borrelia species are causal agents of relapsing fever and are transmitted from human to human by lice or ticks. Relapses during disease are associated with antigenic variation of the spirochete during the infectious disease process. Tetracycline and chloramphenicol are the drugs of choice in treatment.

Leptospira species pathogenic for humans are harbored by rodents. Transmission is through ingestion or contact with contaminated water. Leptospirosis may affect many different organs, but the most serious form of the disease, called infectious jaundice, is associated with liver cell destruction. Penicillin and streptomycin are effective during the early stages of the disease.

A newly recognized disease called Lyme disease is associated with a tick. The organism is classified as a *Borrelia*, but diagnosis is based solely on clinical grounds.

Spirillum minor is a flagellated organism that is the cause of rat-bite fever. The organism has not been cultivated in the laboratory. The lesion is localized on the skin, but the organism can penetrate the blood to produce undulating fever. Penicillin is used in treatment.

Campylobacter is a flagellated bacterium that is found in many animals and is secondarily transmitted to humans. The agent of disease in humans is *C. fetus* and is composed of two subspecies, *jejuni*, which causes diarrheal disease, and *intestinalis*, a cause of disease in compromised patients. If antibiotic treatment is required erythromycin is the drug of choice.

QUESTIONS FOR STUDY

1. What is Lyme's disease? How is it transmitted?
2. In which stages is syphilis contagious?
3. What are the distinguishing symptoms of syphilis during the primary, secondary, latent, and tertiary stages?
4. How does the motility of spirochetes differ from that of the curved rods?
5. How does one distinguish between *Treponema*, *Borrelia*, and *Leptospira*?
6. How are infants affected by congenital syphilis?
7. What microscopical methods can be used to diagnose syphilis? During what stage or stages can they be used?
8. What is the mechanism of attachment of treponemes to host tissue?
9. Under what laboratory conditions do biological false-positives for syphilis occur?
10. What is the most commonly used serological test for syphilis? How does it operate?
11. What is the Jarisch-Herxheimer reaction? What are the conditions in which it occurs?
12. What is responsible for the relapsing fever associated with infection by *Borrelia* species?
13. Infectious jaundice is caused by which microbial species? Which organs are affected?
14. What type of disease is caused by *Campylobacter fetus* subspecies *jejuni*? How could this disease be prevented?
15. What is the causal agent of rat-bite fever? How is the organism cultivated in the laboratory?

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29. MYCOPLASMAS AND L-FORMS

OBJECTIVES

To describe the differences between mycoplasmas and L-forms

To outline the mechanism of transmission, symptoms, and treatment for *Mycoplasma pneumoniae* infection

To understand the significance of the mycoplasmas as agents of sexually transmitted disease

General Characteristics of the Mycoplasmas
General Characteristics of L-Forms
Pathogenesis
Mycoplasma pneumoniae
Other Mycoplasmas and L-Forms
Laboratory Diagnosis
Treatment and Prevention
Other Mycoplasmas and Mycoplasma-like Organisms

The mycoplasmas are a unique group of bacteria in that they lack a cell wall and yet are free-living. They can be found in animals, humans, soil, and plants. Until the late 1930s many investigators referred to them as small bacteria or even viruses because of their ability to pass through bacterial filters. The causal agent of bovine pleuropneumonia, for example, was at first believed to be a virus called PPO (pleuropneumonia organism). In the late 1930s it was shown to be a filamentous bacterium that fragmented into spherical particles. Other microorganisms with similar morphological characteristics were later isolated from human and animal sources and collectively classified as PPLO (pleuropneumonia-like organisms). The mycoplasmas have now been classified in the class Mollicutes, within which are three families: Mycoplasmataceae, Acholeplasmataceae, and Spiroplasmataceae. Only the Mycoplasmataceae contain important human pathogens. The Spiroplasmataceae are plant pathogens. Two genera in the family Mycoplasmataceae are *Mycoplasma* and *Ureaplasma*, the latter's distinguishing characteristic being the ability to hydrolyze urea. Three mycoplasmas cause disease in humans—*M. pneumoniae* (*atypical pneumonia*), *M. hominis* (upper urinary tract disease), and *Ureaplasma urealyticum* (nongonococcal urethritis).

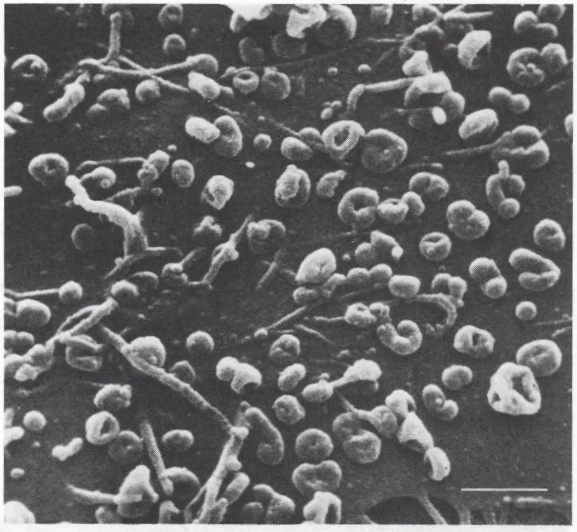
At about the same time that the mycoplasmas were first being described, it was observed that colonies of *Streptobacillus moniliformis* resembled, in appearance and structure, the colonies of *Mycoplasma*. The cells in the *S. moniliformis* colonies lacked cell walls and could not be stained by the usual methods. These cells, now called *L-* (Lister) *forms*, are variants of the cell wall–containing parent cell. They differ from mycoplasmas in that they are capable of reverting back to the typical parental form. L-forms have now been found in most species of bacteria.

GENERAL CHARACTERISTICS OF THE MYCOPLASMAS

M. mycoides is the causative agent of bovine pleuropneumonia and is the prototype of the *Mycoplasma* agent. Mycoplasmas can assume a variety of morphological forms such as coccoid, filamentous, or cocci in chains because they lack a cell wall determinant. The small coccoid units vary in diameter from 0.2 to 0.3 μm in diameter. Some of the different morphological forms are illustrated in Figure 29-1. Although lacking a cell wall the mycoplasmas are stable in ordinary culture media apparently because of the presence of sterols in the cytoplasmic membrane. These sterols are not found in any other bacterial cells or their L-forms. Electron microscopical examination of the cytoplasmic membrane has revealed some portions to be embedded with particles (Fig. 29-2). The particle-free areas appear to be involved in the process of attachment to epithelial cells (see Pathogenesis).

Most *Mycoplasma* species are aerobes or facultative anaerobes, although a few species require anaerobic conditions for initial isolation in the laboratory. For laboratory cultivation the mycoplasmas need a basal medium of meat infusion and peptone supplemented with horse serum or ascitic fluid. The serum or ascitic fluid supplies the fatty acids or lipid precursors required by many species. The medium is usually supplemented with penicillin and thallium acetate to inhibit the growth of any bacterial contaminants. Most mycoplasmas exhibit

Fig. 29-1. Electron micrograph of a species of *Mycoplasma* showing different morphological forms. (Courtesy of E. S. Boatman.)



optimal growth at temperatures between 30° and 36°C in media at alkaline pH's. *Ureaplasma* exhibits optimal growth at acid pH's and is capable of hydrolyzing urea, a property useful in distinguishing it from other mycoplasmas.

Cultivated on agar, colonies of mycoplasmas appear transparent to the naked eye. Closer examination with a hand lens or microscope reveals a colony with a dense central area and a transparent periphery. The dense central portions of the colony may penetrate the agar surface. Overall the colony looks something like a fried egg (Fig. 29-3).

GENERAL CHARACTERISTICS OF L-FORMS

The L-form is a wall-deficient bacterial variant that may occur spontaneously in a bacterial culture or may be induced by certain agents. Two of the agents used to induce transformation are penicillin and ultraviolet light.

Fig. 29-2. Electron micrograph (× 110,000 before 20% reduction) of cross fracture of *Mycoplasma pneumoniae*. Bar = 0.25 nm. Note membrane-embedded particles (*arrow*). Particle-free areas may be associated with attachment process. (From F. Wall, et al., *J. Bacteriol.* 154:924, 1983.)

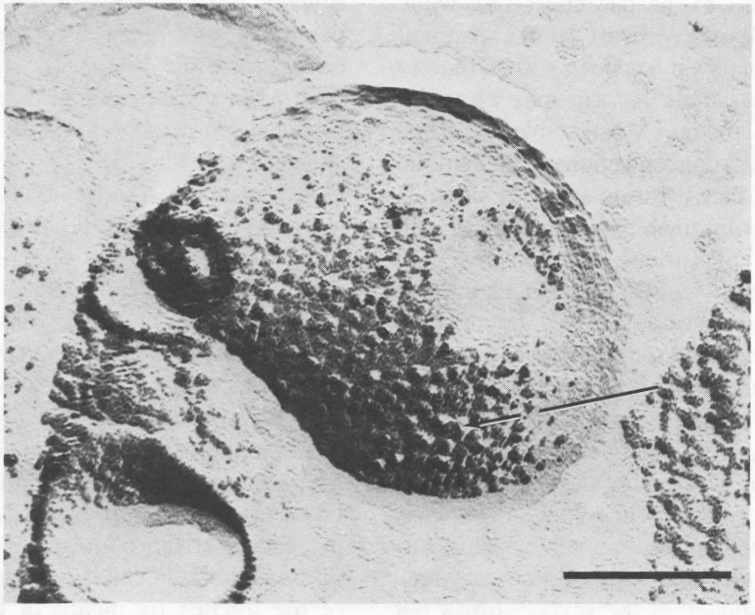
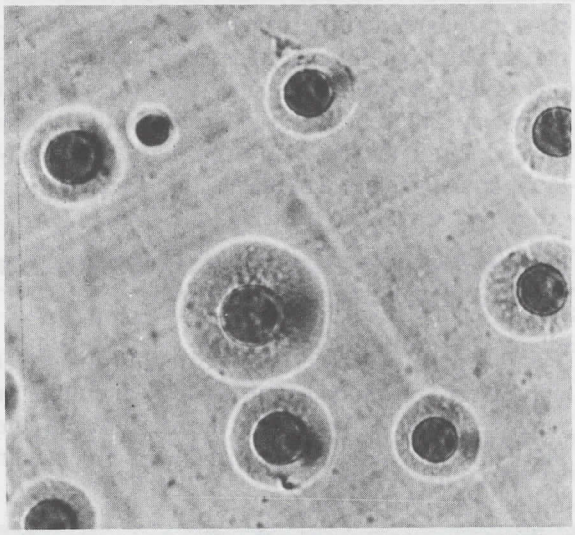


Fig. 29-3. Colony of *Mycoplasma salivarium* showing typical umbonate (button-like) or fried egg shape. Colonies are 0.1 mm in diameter. (From P. F. Smith, *The Biology of the Mycoplasmas*. New York: Academic, 1971.)



In a population of L-forms some are stable (that is, they fail to revert to the parental type), while others are unstable (they revert to the parental type). In a few instances it has been shown that by proper adaptation a stable L-form may be maintained on isotonic media. Most stable L-forms, however, require high concentrations of an osmotic stabilizer. The fact that L-forms are without walls does not mean they are unable to produce cell wall products. L-forms of various gram-positive and gram-negative bacteria can produce wall biosynthetic intermediates. For example, lipoteichoic acid, which binds cell wall to the cell membrane, is formed by an L-form of *Streptococcus pyogenes*. The endotoxin of such gram-negative species as *Proteus* and *Salmonella* is produced, albeit in reduced quantities, by L-forms of these genera. The inability of the L-forms to form complete cell walls may be due to some conformational change in surface geometry or to a lack of appropriate enzymes. The L-forms are similar to the mycoplasmas but differ in the following ways:

1. L-forms require a high concentration of salt in the medium to maintain cellular integrity. The mycoplasmas do not.
2. L-forms can revert to the stable bacterial parental type. Mycoplasmas do not revert to any other bacterial form.
3. L-forms do not have a high lipid content or sterols in the membrane as do the mycoplasmas.
4. Reproduction of L-forms is inhibited in the presence of penicillin. Penicillin has no effect on the mycoplasmas.
5. The guanine-cytosine content of L-forms is the same as that of the bacterial species they were derived from. Most mycoplasmas have a much lower guanine-cytosine content than do other bacterial species.

PATHOGENESIS

MYCOPLASMA PNEUMONIAE

M. pneumoniae produces a human disease called *atypical pneumonia*. This organism infects the upper and lower respiratory tract and usually produces epidemic disease in confined populations such as in military barracks and institutions. The organism is usually transmitted by coughing or sneezing.

Atypical pneumonia (walking pneumonia) is slow to develop, and the incubation period for the disease may be up to 3 weeks. The disease is usually mild and self-limiting with infrequent complications. Symptoms include fever, headache, and a persistent nonproductive cough.

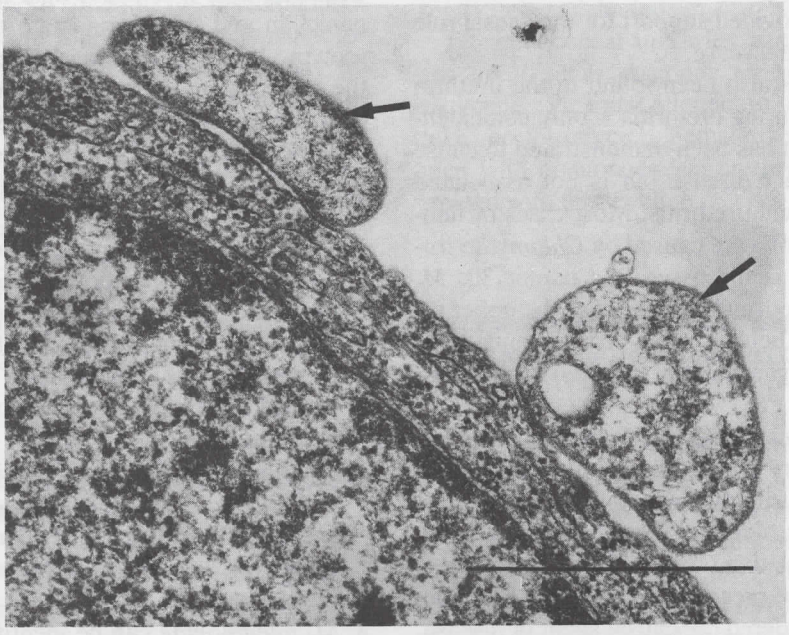
The mechanisms of virulence of *M. pneumoniae* have received considerable attention in the past 15 years, but no absolutely conclusive results have been obtained. Experimental studies with tracheal tissue in organ culture have revealed some interesting details of the pathogenic mechanisms. Mycoplasmas are believed to attach to the ciliated cells of the trachea by means of the microorganisms'

Fig. 29-4. Electron micrograph of *Mycoplasma gallisepticum*. Blebs (*arrows*) seen at the tips of the organism may be involved in motility. (From S. Razin, et al., *Infect. Immun.* 30:538, 1980.)

constricted ends, which appear as blebs (Fig. 29-4). These blebs correspond to the particle-free areas of the microbial cytoplasmic membrane discussed earlier. Unless the microorganism is lodged between dense areas of cilia, the mycoplasmas lie along the membrane surface with large areas in close contact with the host cell (Fig. 29-5). The receptor site on the epithelial surface is believed to be a glycoprotein that is sensitive to neuraminidase. The chemical nature of the binding site on the microbial surface has not been determined but is probably a protein. *Mycoplasma pneumoniae* strains that have lost the ability to adsorb to epithelial cells also lose the ability to produce pneumonia in hamster experimental models. Once the organisms are adsorbed to the cilia, epithelial cell injury, vacuolization, nuclear swelling, and loss of cilia ensues. The mechanism by which epithelial cells are injured is not known.

The level of immunity acquired after infection by *M. pneumoniae* is related to the intensity of the dis-

Fig. 29-5. Electron micrograph of a section of HeLa cell infected with *Mycoplasma arginini*. Two mycoplasmas (*arrows*) are seen adjacent to cytoplasmic membrane. Bar = 1 μm. (Courtesy E. S. Boatman.)



ease. Immunity lasts longer after pneumonia than after mild infections. Even so, immunity after pneumonia is short-lived and lasts no more than 7–8 years. There is a decreasing attack rate after the age of 10, which represents an acquired immunity from early childhood disease. Immunity is probably built up after the age of 10 by repeated subclinical disease.

OTHER MYCOPLASMAS AND L-FORMS

Ureaplasma urealyticum and *M. hominis* are mycoplasmas that colonize both the male and the female genital tract. Colonization occurs initially during birth as the infant passes through the birth canal. The rate of isolation of these organisms appears to decline after birth but increases in both sexes after puberty with increased sexual activity. There is an apparent decline in genital mycoplasmas after menopause. The importance of these organisms in disease has been difficult to evaluate. *U. urealyticum* plays a role in the disease nongonococcal urethritis. This organism has been isolated in increased numbers during urethritis. Intraurethral inoculation of this organism into chimpanzees as well as humans has provided support for the causal role of this organism in disease.

M. hominis has also been found in the urethra, but its isolation during urethritis is only coincidental. This organism has been demonstrated to cause upper urinary tract disease but is not associated with nongonococcal urethritis. Most cases of nongonococcal urethritis are caused by *Chlamydia trachomatis*, which is discussed in Chapter 30. *M. hominis* is also a normal inhabitant of the upper respiratory tract. There is some circumstantial evidence that this organism may be a cause of exudative pharyngitis.

The mycoplasmas have also been implicated in other genitourinary tract diseases such as chronic prostatitis, pelvic inflammatory disease, and septic abortion. The causal role of these organisms in disease is only speculative.

There is only one recorded instance in which an L-form was considered the causal agent of disease. Most information on the pathogenicity of L-forms is circumstantial. It has been suggested that the L-form is a survival mechanism for the bacterial cell. For example, under adverse environmental conditions such as the presence of an antibiotic that inhibits cell wall synthesis, the cell could maintain its viability by remaining as an L-form until environmental conditions improved. In the L-form state some pathogenic mechanisms could still be in operation. Studies have shown that L-form gram-negative species produce endotoxin; L-forms of *S. pyogenes* produce a lipoteichoic acid that mimics the destructive effect of intact microorganism on heart, kidney, and liver tissue; L-forms of *Hemophilus parainfluenzae* cause a pneumonia in pigs that is indistinguishable from that produced by natural infection with *H. parainfluenzae*.

LABORATORY DIAGNOSIS

Isolation of mycoplasmas from clinical material is accomplished by swabbing infected areas and inoculating onto a selective agar medium containing penicillin and thallium acetate to inhibit bacterial growth. In 1 week microscopical examination of the colonies can be made by inverting the culture dish and looking for the characteristic fried egg appearance. Identification of specific *Mycoplasma* species may require the following techniques:

1. Filter disks containing a growth-inhibiting antiserum against specific *Mycoplasma* species are the principal recommended identification technique. After incubation for 1 week a positive identification is indicated as a clear zone around the disk as compared to a control disk containing normal serum.
2. *M. hominis* colonies are differentiated from *Ureaplasma* colonies by adding urease reagent to the colonies. *Ureaplasma* colonies turn brown. *M. hominis* strains are unaffected by urease reagent.
3. *M. pneumoniae* can be identified on blood agar

by its ability to produce beta hemolysis. All other species exhibit alpha or gamma hemolysis.

4. *M. pneumoniae* can be identified by its ability to reduce tetrazolium salts that have been incorporated into the agar. The area under the colonies appears pink.
5. *M. pneumoniae* colonies are flooded with sheep erythrocytes and then stained. The erythrocytes coat the colonies of *M. pneumoniae* but not those of other species. They can be observed microscopically.

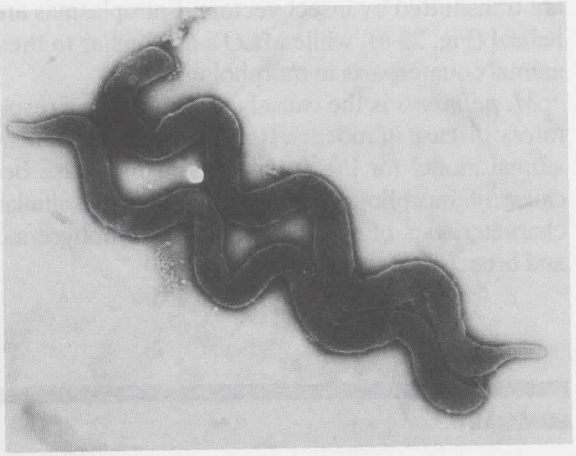
Culturing as a means of identification of mycoplasmas is time-consuming; it may take 2–3 weeks. Serological tests such as the complement fixation test are recommended for rapid presumptive diagnosis, but these tests are laborious and not very sensitive. A test that appears to be specific for the direct identification of mycoplasmas in tissue, called the *immunoperoxidase test*, can be applied to nasal or conjunctival swabs as well as tissue. The test is much easier to interpret than other staining methods including fluorescent antibody.

The isolation and identification of L-forms has never been part of the usual regimen in the clinical laboratory and is more suited to the research laboratory. If L-forms are found to be responsible for certain infections, the clinical laboratory may require, in addition to the usual media for isolation of the natural agent, special hypertonic media. Some studies have shown that when both types of media are used a greater percentage of positive cultures is obtained. Thus some so-called negative specimens may actually be positive because of the presence of L-forms. The implications could be important if the individual is a carrier of a very contagious agent such as *Neisseria gonorrhoeae*.

TREATMENT AND PREVENTION

Erythromycin as well as several tetracyclines are effective in the treatment of mycoplasmal pneumonia. Erythromycin is preferred in the treatment of children because of the risk of staining immature teeth when tetracyclines are used over prolonged periods of time.

Fig. 29-6. Electron micrograph (×57,600 before 48% reduction) of honeybee *Spiroplasma*. (Courtesy R. G. Cole.)



Formalin-killed vaccines have been used with some success in the military. Live vaccines are currently being evaluated.

Nongonococcal urethritis caused by *Ureaplasma urealyticum* responds to treatment with tetracycline. Patients allergic to tetracyclines may take erythromycin. Female sexual partners of men with nongonococcal urethritis should also be treated with tetracycline.

OTHER MYCOPLASMAS AND MYCOPLASMA-LIKE ORGANISMS

Mycoplasmas are ubiquitous organisms that are causal agents of disease in a variety of animals, plants, and insects. Mycoplasmas are associated with chronic arthritis in animals, and it has been suspected that they are associated with the same condition in humans. *M. fermentans* has been isolated from the synovial fluid of many patients with

rheumatoid arthritis, but the organism has not been cultivated directly from this source.

Spiroplasma citri and mycoplasma-like organisms (MLO's) are important plant pathogens that are transmitted by insect vectors. Spiroplasmas are helical (Fig. 29-6), while MLO's are similar to their animal counterparts in morphology.

M. pulmonis is the causal agent of chronic respiratory disease in rodents. Rats provide an excellent animal model for the study of human disease because of morphologic similarities and the similar characteristics of chronic bronchitis, emphysema, and bronchiectasis.

SUMMARY

Mycoplasmas are free-living bacteria that do not possess a cell wall. L-forms are wall-less forms of bacterial species that may occur spontaneously or may be induced by certain chemical agents.

Mycoplasmas pathogenic for humans belong to the family Mycoplasmataceae, which contains two genera, *Mycoplasma* and *Ureaplasma*. Members of *Mycoplasma* possess sterols in the cytoplasmic membrane that lend stability to the microbial cell. *Mycoplasma pneumoniae* is the causal agent of atypical pneumonia, a relatively mild disease occurring in confined populations of humans. The organism attaches to ciliated cells of the respiratory epithelium by means of constricted ends. The receptor site on the epithelial surface is probably a glycoprotein.

Some mycoplasmas such as *Ureaplasma urealyticum* colonize the genital tract of men and women. They are believed to be a cause of nongonococcal urethritis and may also be involved in other diseases of the genitourinary tract.

L-forms are not considered to be of any pathogenic significance, but they may represent a state of resistance to antibiotics that inhibit the cell wall.

Mycoplasmas take at least a week to produce colonies on agar. Their identification and differentiation from other related species is dependent on serological as well as biochemical tests.

Tetracycline and erythromycin have been used successfully in the treatment of disease caused by *Mycoplasma* and *Ureaplasma*.

QUESTIONS FOR STUDY

1. What is the mechanism by which *M. pneumoniae* adheres to respiratory epithelium?
2. What is the role of *Ureaplasma* in human disease?
3. Why are penicillins not effective against *Mycoplasma* and L-forms? What antimicrobials are effective against them?
4. How could L-forms be important in the infectious disease process?
5. How can L-forms be distinguished from *Mycoplasma*?

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30. RICKETTSIAE AND CHLAMYDIAE

OBJECTIVES

To outline the differences and similarities between the rickettsiae and chlamydiae

To list the causative agent, method of transmission, pathogenesis, symptoms, and treatment for Rocky Mountain spotted fever, epidemic typhus, Q fever, and trench fever

To describe the types of infections that can be caused by *Chlamydia trachomatis* and by *C. psittaci*

To list the methods of treatment and prevention for chlamydial disease

The Rickettsiae
General Characteristics
Pathogenesis
Typhus Group
Epidemic or Louse-borne Typhus
Endemic Typhus
Spotted Fever Group
Rocky Mountain Spotted Fever
Boutonneuse Fever
Asian Tick Fever
Rickettsialpox
Scrub Typhus
Q Fever
Trench Fever
Laboratory Diagnosis
Treatment, Prevention, and Control
The Chlamydiae
General Characteristics
Pathogenesis
Chlamydia trachomatis
Ocular Disease
Male Genital Tract Disease
Female Genital Tract Disease
Lymphogranuloma Venereum
Chlamydia psittaci
Laboratory Diagnosis
Treatment and Prevention

The rickettsiae and chlamydiae are both intracellular parasites (*Rochalimaea quintana* is the only species that can be cultivated on ordinary laboratory media) that are found in invertebrate and vertebrate hosts, including humans. Although originally believed to be viruses because of their size and difficulty of cultivation, they are now recognized morphologically and biochemically as bacteria. There are many similarities between the rickettsiae and the chlamydiae. The chlamydiae, however, are easily distinguished from the rickettsiae because of their unique developmental cycle. In addition, none of the chlamydiae are transmitted by insect vectors, whereas nearly all rickettsial species are transmitted by insect vectors.

THE RICKETTSIAE

GENERAL CHARACTERISTICS

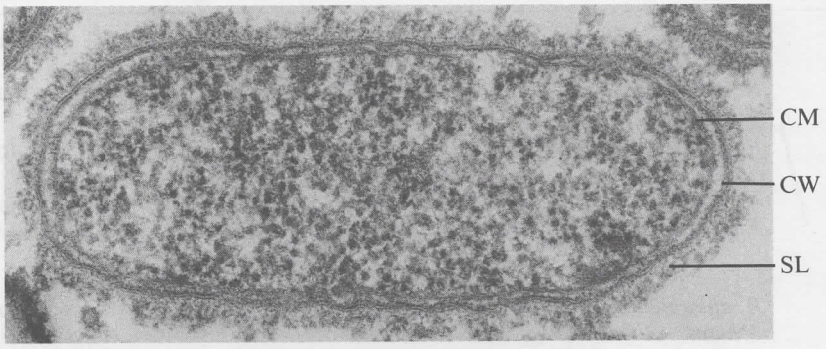
The rickettsiae are very small gram-negative rod-shaped bacteria that were at one time all included in the genus *Rickettsia* but because of variation in structure and metabolism have been divided into three genera: *Rickettsia, Coxiella*, and *Rochalimaea*. *Rochalimaea*, unlike the other two genera, is an extracellular parasite. The typical rickettsia is approximately 0.3 to 0.7 μm in diameter and 2.0 μm in length. It possesses the typical three-layered cell wall associated with other gram-negative bacteria (Fig. 30-1). Some species possess an extracellular slime layer. The rickettsiae stain poorly in the Gram-staining technique and are best observed in preparations stained by the Giemsa or Macchiavello methods.

Rickettsiae can be cultivated in the yolk sac of embryonated eggs, in laboratory animals, in certain arthropods, and in some tissue cultures. Removed from the intracellular environment of the host cell, rickettsiae are susceptible to inactivation by heat, drying, and chemicals. They divide by transverse fission. Under poor nutritional conditions, the rod-shaped cells develop into long filaments several micrometers in length. In a rich, growth-supporting medium the filaments divide into typical rods.

Metabolically, the rickettsiae have a tricarboxylic acid cycle, an electron transport system, and the enzymes essential to biosynthesis of macromolecules. Why they require an intracellular habitat is still a mystery.

All rickettsiae are sensitive to the antibiotic tet-

Fig. 30-1. Electron micrograph (×69,500) of *Rickettsia rickettsii*. The cell envelope is typical of a gram-negative bacterial cell wall. (CM = cytoplasmic membrane; CW = cell wall; SL = slime layer.) (From D. J. Silverman and C. L. Wisseman, Jr., *Infect. Immun.* 21:1020, 1978.)



racycline, as would be expected from their prokaryotic nature.

PATHOGENESIS

With the exception of *Coxiella burnetii*, the causal agent of Q fever, the rickettsiae are transmitted to humans exclusively by insect vectors: ticks, mites, lice, and fleas. In ticks and mites the rickettsiae multiply in the salivary glands and can be transferred to the host by biting. In fleas and lice the rickettsiae multiply in the gut and are excreted in the feces. Transfer of rickettsiae from fleas and lice to a human host occurs by crushing of the arthropod or by arthropod defecation at the site of the bite.

In general the pathological features of all rickettsial diseases differ only in the severity of the clinical symptoms. Once the rickettsiae have penetrated the skin, they quickly reach the bloodstream (rickettsemia). In the vascular system they have an affinity for the endothelial cells of small blood vessels. The infected endothelial cells become detached from the blood vessels and in the bloodstream can bring about vascular obstruction that eventually leads to tissue necrosis. Many of the symptoms of rickettsial infection—rash, myocarditis, and neurological changes—are due to rickettsiae in the vascular tissue of such organs as the skin, heart, and brain, respectively. At the site of the arthropod bite, an encrusted ulcer (*eschar*) may appear. Later during the disease, the ulcer develops a black necrotic center. The eschar is not found in all rickettsial diseases, but when it is it provides a diagnostic clue.

The rickettsiae differ in the manner they interact with the host cell. Initially the rickettsiae are actively phagocytosed, a condition which is necessary for infection, but to become successful parasites they must get out of the phagolysosome (except for *Rochalimaea quintana*). It appears that the *Rickettsia* possess a *phospholipase A* that breaks down the membrane of the phagolysosome. Once released from the phagolysosome the *Rickettsia* divide rapidly in the cell cytoplasm or within the nucleus of the phagocytic cell. As their numbers increase in the phagocyte, some *Rickettsia* species are released to infect other cells by breakdown of the cytoplasmic membrane. Other *Rickettsia* species are released without breakdown of the cytoplasmic membrane. *Coxiella burnetii* grows in the cytoplasm of the cell enclosed within vacuoles (not phagolysosomes). The vacuoles eventually fuse to form a single large vacuole that occupies most of the volume of the cell.

The rickettsial diseases of humans can be classified into five major groups: the typhus group, the

Table 30-1. Human Rickettsial Diseases

Group	Causative agent	Common name	Mode of transmission to humans	Reservoir of rickettsiae
Typhus	*Rickettsia prowazekii*	Epidemic typhus	Louse	Human
	R. typhi (mooseri)	Endemic or murine typhus	Flea	Rodents
Spotted fever	*R. rickettsii*	Rocky Mountain spotted fever	Tick	Rodents
	R. siberica	Asian tick fever	Tick	Rodents
	R. conorii	Boutonneuse fever, African tick typhus	Tick	Rodents
	R. australis, R. akari	Rickettsialpox	Mite	Marsupials or mice
Scrub typhus	*R. tsutsugamushi*	Scrub typhus	Mite	Rodents
Q fever	*Coxiella burnetii*	Q fever	Contact with infected cattle or their parturition products	Cattle, sheep, or goats
Trench fever	*Rochalimaea quintana*	Trench fever	Louse	Humans

spotted fever group, scrub typhus, Q fever, and trench fever (Table 30-1).

Typhus Group

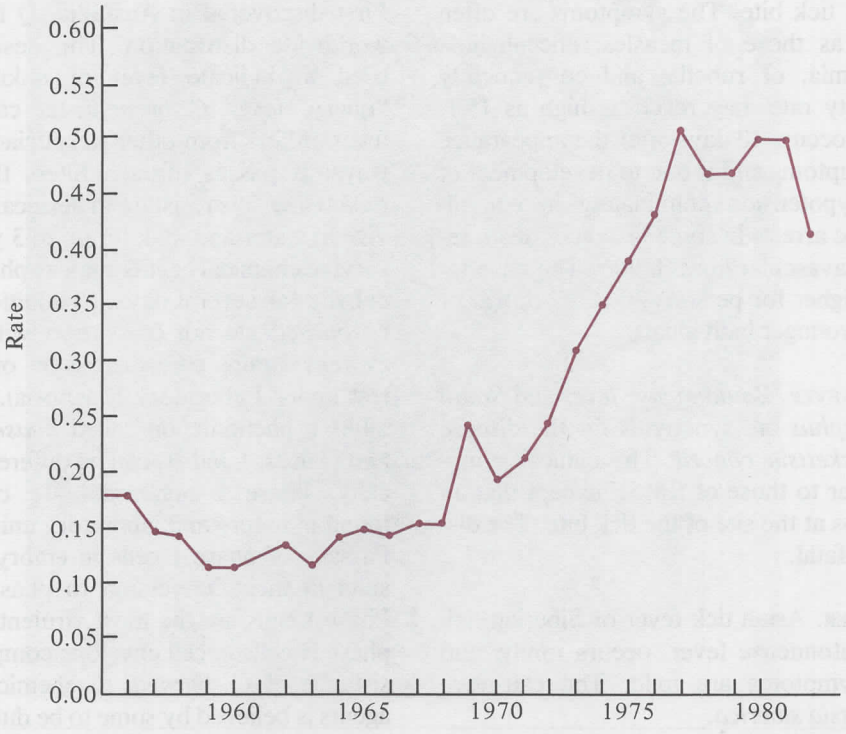
EPIDEMIC OR LOUSE-BORNE TYPHUS. Louse-borne typhus is caused by *Rickettsia prowazekii* and is transferred to humans by the body louse. The organisms are passed in the louse feces during biting and enter the wound around the bite. Before the discovery and use of DDT, severe epidemics of louse-borne typhus were common during times of war and famine. The disease today is no longer a major public health problem and appears to be confined to certain areas of Africa and Asia, where it is still an important cause of illness. The fatality rate of epidemics can run as high as 30 percent. In areas where socioeconomic standards are low and body lice are prevalent, epidemics remain a constant threat to the population.

Thirty cases of illness caused by *R. prowazekii* were documented in the United States from 1976 to 1983. These cases were unusual in that they were sporadic. In several cases flying squirrels or nests of flying squirrels were found in the home of the patient. Flying squirrels often nest in attics during the winter months, and 75 percent of the cases of disease occurred during the winter months. The mechanism of transmission of the rickettsial agent is not known.

The incubation period of louse-borne typhus lasts 10–14 days and is characterized by a high fever, which tends to remain during the course of the illness. On the fourth or fifth day a rash appears on the trunk and spreads to the extremities. The rash is seldom seen on the face, palms, or soles. Death is the result of myocardial or neurological involvement.

In some individuals who have recovered from an initial attack of epidemic typhus, surviving rickettsial microorganisms may enter a latent stage. The infected carrier can develop disease in the future but will not have the characteristic rash. The carrier state has been referred to as *Brill-Zinsser disease*.

Fig. 30-2. Reported cases of Rocky Mountain spotted fever, United States, 1955–1982. (From Centers for Disease Control.)



ENDEMIC TYPHUS. Endemic or murine typhus is a mild form of typhus transmitted to humans by the rat flea. The causative agent is *Rickettsia typhi* (*R. mooseri*). The symptoms of endemic typhus are similar to those of epidemic typhus but are much milder. Immunity to infection by epidemic typhus also confers immunity to endemic typhus.

As many as 5000 cases of murine typhus were reported in the United States in the 1940s. Today less than 100 cases are reported annually to the Centers for Disease Control, with most of these reported from Texas.

Spotted Fever Group

ROCKY MOUNTAIN SPOTTED FEVER. Of reported rickettsial diseases in the United States, 90 percent are Rocky Mountain spotted fever (RMSF). RMSF is primarily a disease of children and young adults. More than 60 percent of all cases occur in persons younger than 20 years old. Since 1960 there has been a 200 percent increase in the number of reported cases (Fig. 30-2). The causative agent is *Rickettsia rickettsii*. Most cases are found east of the Mississippi, particularly in Virginia, North Carolina, and Tennessee. In the eastern United States the American dog tick, the primary vector in transmission of the rickettsiae to humans, is harbored by the rabbit, raccoon, fox, woodchuck, and deer. The wood tick is responsible for the transmission of RMSF in the western United States.

Clinical symptoms, which appear on the average 2–5 days after the tick bite, include fever, head-

ache, lymphadenopathy, and rash. The rash generally starts on the wrists and then spreads to the trunk, face, and extremities. In contrast with other rickettsial infections, an eschar seldom develops at the site of the tick bite. The symptoms are often misdiagnosed as those of measles, encephalitis, meningococcemia, or rubella, and consequently the case fatality rate may reach as high as 15:1. Death usually occurs 10 days after the appearance of the first symptoms and is due to development of progressive hypotension culminating in convulsions or cardiac arrest. In some instances death results from intravascular coagulation. The case fatality rate is higher for persons 30 years of age or older than for younger individuals.

BOUTONNEUSE FEVER. *Boutonneuse fever* and *South African tick typhus* are synonyms for the disease carried by *Rickettsia conorii*. The clinical symptoms are similar to those of RMSF except that an eschar develops at the site of the tick bite. The disease is seldom fatal.

ASIAN TICK FEVER. Asian tick fever or Siberian tick fever, like boutonneuse fever, occurs rarely, and the clinical symptoms are mild. The causative agent is *Rickettsia siberica*.

RICKETTSIALPOX. Rickettsialpox is the mildest of all rickettsial diseases. It has a worldwide distribution and is caused by *Rickettsia australis* or *Rickettsia akari*. The vector for transmission is the mite.

Scrub Typhus

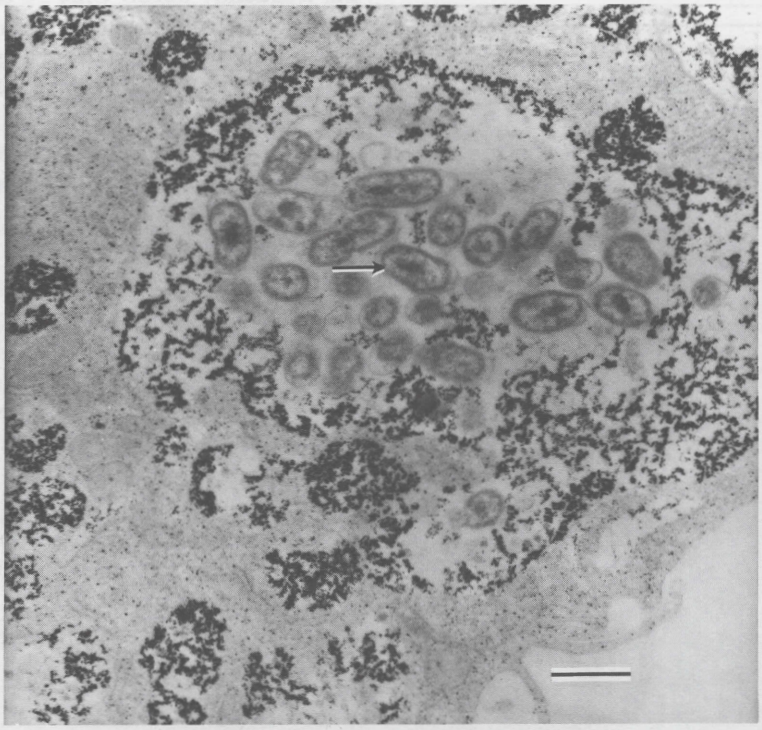
Scrub typhus was first recognized during the sixteenth century in China. It is caused by *Rickettsia tsutsugamushi* and is transmitted to humans by mites carried on rodents. Today the disease is more prevalent in eastern Asia and the islands of the South Pacific. Clinical symptoms include a maculopapular rash that covers most of the body and an eschar at the site of the mite bite. Enlargement of the spleen occurs in more than 50 percent of the cases. Treatment with antibiotics brings complete recovery; untreated, the disease progresses until there are serious respiratory, neurological, and cardiovascular complications, often leading to death.

Q Fever

First discovered in Australia, Q fever now has a worldwide distribution. The designation *Q* was used to indicate fever of unknown origin or "query" fever. *C. burnetii*, the causal agent of Q fever, differs from other rickettsiae in a number of ways: It passes through filters that retain other rickettsiae, it is resistant to desiccation and can survive in water and milk for up to 3 years, and it can survive chemical agents such as phenol and formaldehyde for several days. In addition antibodies to *C. burnetii* do not cross-react with agglutinins of *Proteus* strains (see discussion of the Weil-Felix test under Laboratory Diagnosis). *C. burnetii* exhibits a phenomenon called *phase variation*. The two phases, I and II, can be differentiated serologically. Phase I organisms are characteristically found in nature and laboratory animals (Fig. 30-3). Passage of phase I cells in embryonated eggs results in their conversion to phase II organisms. Phase I cells are the most virulent and differ from phase II cells in cell envelope composition. The resistance of *C. burnetii* to chemical and physical agents is believed by some to be due to the complex life cycle of the organism in which a large and small form exist. The larger form is believed to give rise to a spore-like cell that germinates and becomes a resistant smaller cell (Fig. 30-4).

C. burnetii infects ticks that are associated with rodents, cattle, and sheep. Humans acquire Q fever by direct or indirect contact with infected animals. The uterus and udder of cattle are often infected, and people may acquire the organisms by drinking contaminated milk or by contact with infected parturition products at the time of animal delivery. Since *C. burnetii* is resistant to drying, the parturition products can contaminate the environment and serve as an indirect source of human infection. Q fever differs from other rickettsial diseases in that there is no rash associated with it and the Weil-Felix reaction (see under Laboratory Diagnosis) is negative. The initial symptoms, fever and persistent cough, often resemble those of a mild case of

Fig. 30-3. Electron micrograph of phase I *Coxiella burnetii* (*arrow*) within a cytoplasmic vacuole. Bar = 1.55 nm. (From O. G. Baca, *Infect. Immun.* 40:1158, 1983.)



influenza. The principal organ involved in infection is the lung, and an atypical interstitial pneumonia develops. Although the disease is self-limiting, antibiotic treatment may be necessary if complications ensue. Endocarditis is a frequent sequela of Q fever in humans with prior heart disease. Where Q fever is endemic among farmers, abattoir and dairy workers, and others who work with livestock, *C. burnetii* should be considered in the etiology if pericarditis is diagnosed.

Trench Fever

R. quintana is the causative agent of trench fever. Human beings are the reservoir of infection, and the body louse is the only known vector. Trench fever was an important clinical entity during the two world wars. Because the symptoms are mild, trench fever is not considered a public health problem.

LABORATORY DIAGNOSIS

Rickettsiae can be detected in smears of infected tissue by staining techniques; however, they cannot be differentiated from other microorganisms. Serological diagnosis is the most practical and efficient method of rickettsial identification. The complement fixation test is the most widely used serological test for identifying these microorganisms. It is designed to detect antibodies and rises in antibody titer during the course of the disease. In addition a number of agglutination tests have been used diagnostically, particularly for epidemic typhus and

Fig. 30-4. Electron micrograph of *Coxiella burnetii* demonstrating the endospore-like component (*arrow*). (From T. F. McCaul and J. C. Williams, *J. Bacteriol.* 147:1963, 1981.)

Q fever. Because of the excessive amounts of rickettsial antigen required for the tests and the difficulty of preparing such quantities, a microagglutination technique has been devised. The agglutination tests are much more sensitive than the complement fixation tests and can distinguish between epidemic and endemic typhus.

The *Weil-Felix test* is another serological test used for diagnosis of all rickettsial diseases except Q fever, trench fever, and rickettsialpox. It is based on the antigenic relationship between strains of the bacterium *Proteus* (OX-19, OX-2, and OX-K) and rickettsiae. *Proteus* strains can be agglutinated by antibodies induced by various rickettsiae. The test is valuable in establishing a presumptive diagnosis of rickettsial disease since nonspecific reactions can frequently occur. Only 50 to 70 percent of patients with scrub typhus show a rise in OX-K agglutinins. In murine and louse-borne typhus most patients are positive for OX-19 agglutinins. For Rocky Mountain spotted fever, Weil-Felix agglutinins are seldom constant.

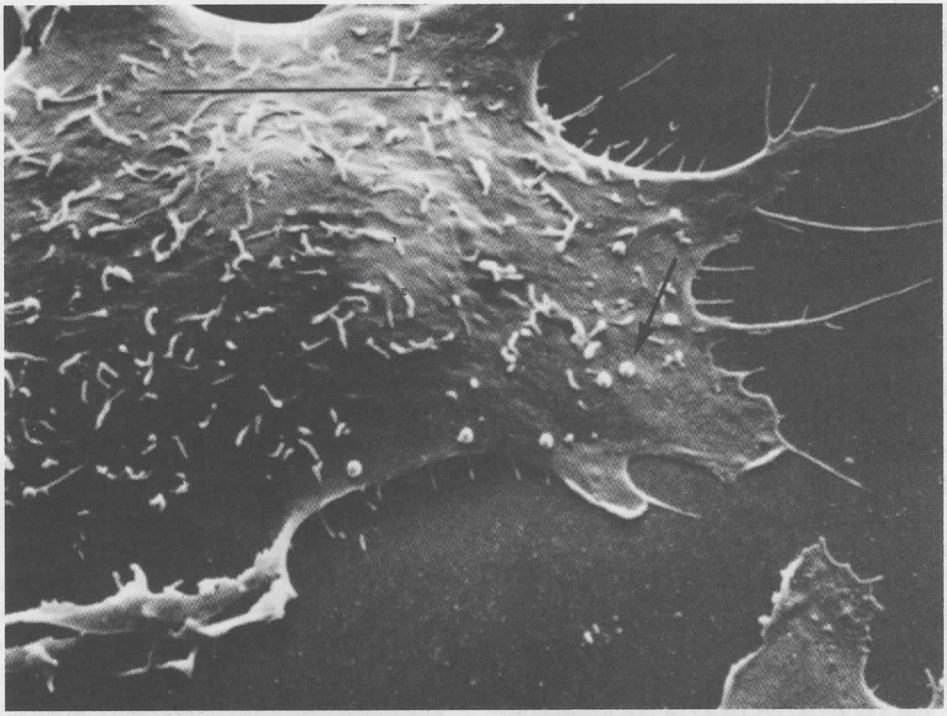
Many of the serological techniques do not yield rapid results because of the time required for production of a sufficient antibody titer in the infected host. A useful serological test is immunofluorescence, which can identify rickettsiae in infected tissue as well as detect rickettsial antibodies. The direct method, relatively inexpensive and simple, uses specific antiserum conjugated with fluorescein isothiocyanate (FITC) to discover rickettsiae in tissue. In the indirect method, which is employed to detect antibody, specific antibody is fixed to rickettsial antigen. The latter complex is allowed to combine with FITC-conjugated antibody against specific antiserum.

C. burnetii is a very contagious agent and can be easily acquired by laboratory personnel if certain precautions are not taken during culturing techniques. For this reason it is much safer to use serological tests such as the complement fixation test.

TREATMENT, PREVENTION, AND CONTROL

Treatment of rickettsial disease with tetracycline or chloramphenicol often results in complete recovery. Because of the toxic effects of chloramphenicol, tetracycline is the preferred drug. In some rickettsial diseases, such as RMSF, early treatment is absolutely necessary. Antibiotic treatment should therefore be initiated on the basis of clinical symptoms and not serological diagnosis since antibodies do not always develop in the early stages of the disease. Many of the fatalities from rickettsial infections have occurred because of misdiagnosis and administration of antibiotics such as penicillin and ampicillin that have no effect on the infecting microorganisms. Early therapy, particularly in scrub

Fig. 30-5. Scanning electron micrograph of *Chlamydia psittaci* interacting with host cell. Arrow points to an elementary body attached to host surface. Bar = 5 nm. (From J. W. Moulder. In D. Schlessinger [Ed.], *Microbiology*. Washington, DC: American Society for Microbiology, 1979.)



typhus, does not allow enough time for immunity to develop, and relapses are possible unless treatment is extended for several weeks.

Since many rickettsial diseases are self-limiting and occur infrequently, vaccination programs have met with little success. A vaccine for Q fever has been developed to prevent shedding of *C. burnetii* in cows. A Q fever vaccine has been available since 1967 for use by laboratory personnel and others who are in contact with the organism. This vaccine is prepared from what we would now call phase II organisms. There has been no vaccine for national distribution. Since 1979 there have been four large-scale outbreaks of Q fever at major teaching universities. A commercial vaccine for RMSF, prepared from yolk sacs of embryonated eggs, was thought to provide protection but was removed from the market in 1978. Since that time no RMSF vaccine has been available in the United States. A new formalin-inactivated vaccine was developed in 1983 and is currently under evaluation.

Chemical control with DDT can be easily applied against fleas and lice since they are closely associated with humans and rats. Control of woodland ticks and mites is more difficult because of the variety of mammals that harbor the arthropod vectors. The most practical protection is the use of repellents and personal cleanliness.

THE CHLAMYDIAE

The taxonomic position of the chlamydiae has been a subject of controversy for years. They were originally thought to be large viruses when they were observed during an outbreak of psittacosis in 1929. Chlamydiae have a unique growth cycle and for this reason have been placed in their own order, Chlamydiales. There is one family, Chlamydiaceae, and one genus, *Chlamydia*, with two species, *C. psittaci* and *C. trachomatis*. Interest in the chlamydiae has increased rapidly since they were discovered in the 1970s to be associated with genital disease.

GENERAL CHARACTERISTICS

The chlamydiae, although intracellular parasites like the rickettsiae, differ morphologically and metabolically from them. Chlamydiae range in size from 0.2 to 1.0 μm, depending on their stage of development in the host cell. The cell envelope of one form possesses hexagonal arrays of subunits, which are absent in the other form. Chlamydiae have no peptidoglycan layer, which is present in rickettsiae, but do have an outer membrane composed almost exclusively of a single species of protein. Chlamydiae rely totally on host energy sources, but they can make all their own DNA, RNA, and protein. They have been referred to as "energy parasites." The genome of the chlamydiae is half as large as the rickettsial genome.

PATHOGENESIS

The chlamydiae are among the most ubiquitous parasites in the animal kingdom. *C. psittaci* is found in most birds and other domestic and wild animals, while *C. trachomatis* is associated only with humans. *C. trachomatis* organisms infect mainly the epithelial cells of the ocular and genital mucosa, while *C. psittaci* preferentially interacts with macrophages. As mentioned previously chlamydiae have a unique developmental cycle in which two morphological forms of the organism exist. One form, termed the *elementary body* (*EB*), is the extracellular parasite (Fig. 30-5); the other, called the *reticulate body* (*RB*), is involved in intracellular development (Table 30-2). The infectious cycle can be broken down into the following stages (Fig. 30-6):

1. The elementary body attaches to a receptor site on the host cell surface and is taken into the cell by a phagocytic process.
2. Within the phagosome there is reorganization of the elementary body and its conversion to a reticulate body.
3. The reticulate body multiplies by binary fission. During this multiplication process the phagosome enlarges and is referred to as an *inclusion body* (Fig. 30-7).
4. Some of the reticulate bodies, instead of multiplying, undergo reorganization and become elementary bodies.
5. Elementary bodies are released from the inclu-

Table 30-2. Properties of Chlamydial Particles

Property	Elementary body	Reticulate body
Size	0.2–0.4 μm	0.6–1.0 μm
Rigid cell wall	Yes	No
Extracellular stability	Yes	No
DNA	Nucleoid	Dispersive
Infective	Yes	No
Induces phagocytosis	Yes	No
Inhibits phagosome fusion with lysosome	Yes	No
Toxic	Yes	No
Metabolic activity	No	Yes
Replication	No	Yes

Source: From J. Schachter and H. D. Caldwell, Chlamydiae. *Annu. Rev. Microbiol.* 34:285, 1980.

Fig. 30-6. Chlamydial life cycle. A. Elementary body (EB) (*arrow*) has been phagocytosed and contains electron-dense body at 2.5 hours after infection. B. EB has differentiated into reticulate body (RB). RB is in process of division (*arrow*) at 12 hours after infection. C. Reticulate bodies (*arrow*) still dividing 20 hours after infection. D. RB's have differentiated into EB's with electron-dense bodies (*arrow*) 30 hours after infection. (From J. W. Moulder, *J. Infect. Dis.* 127:155, 1973.)

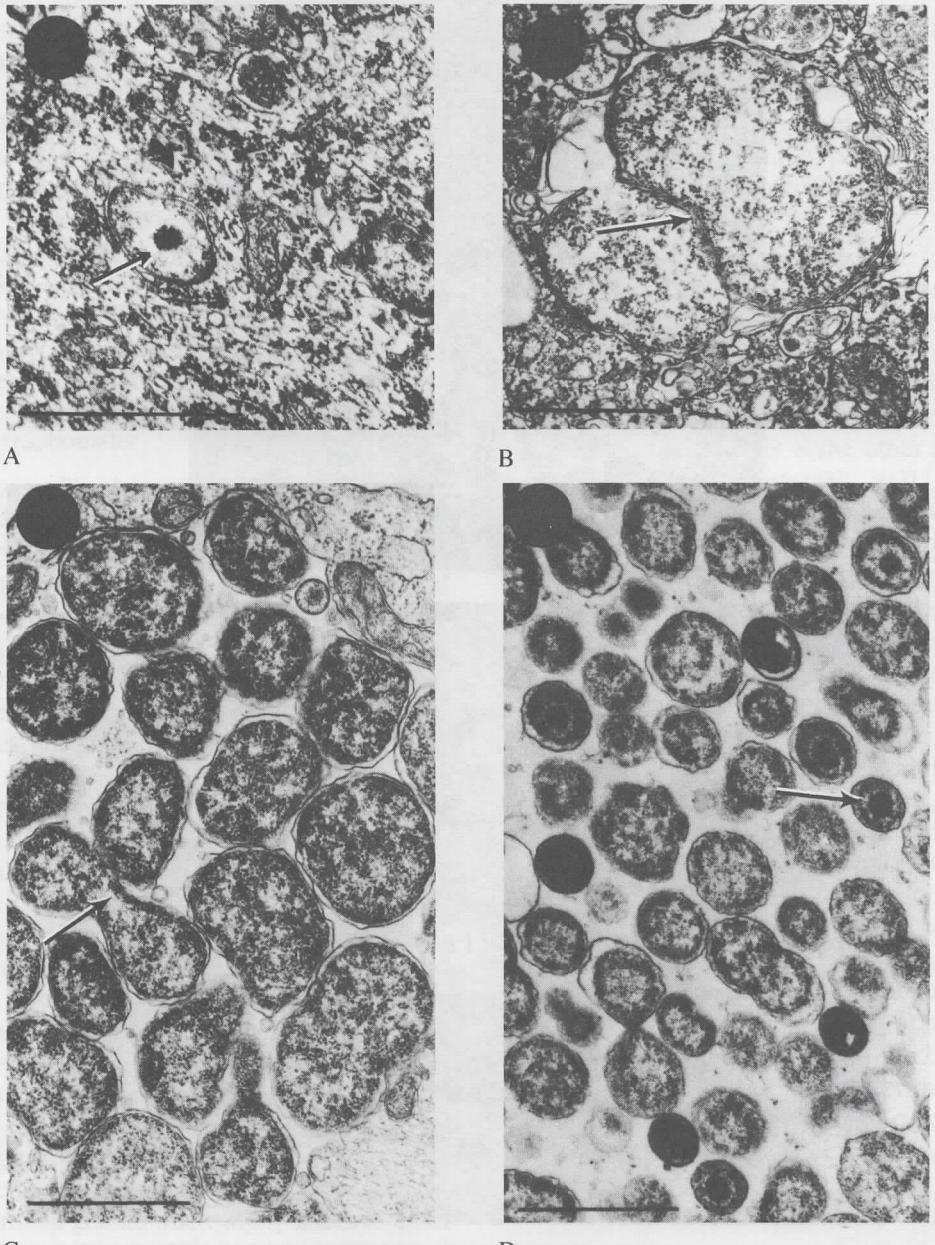
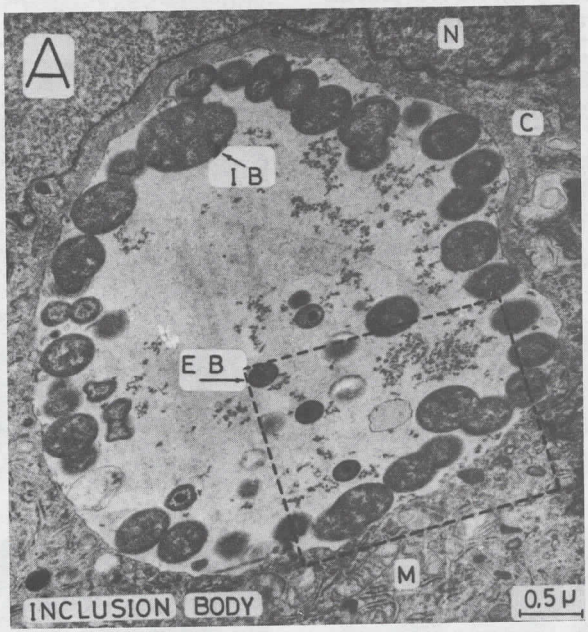
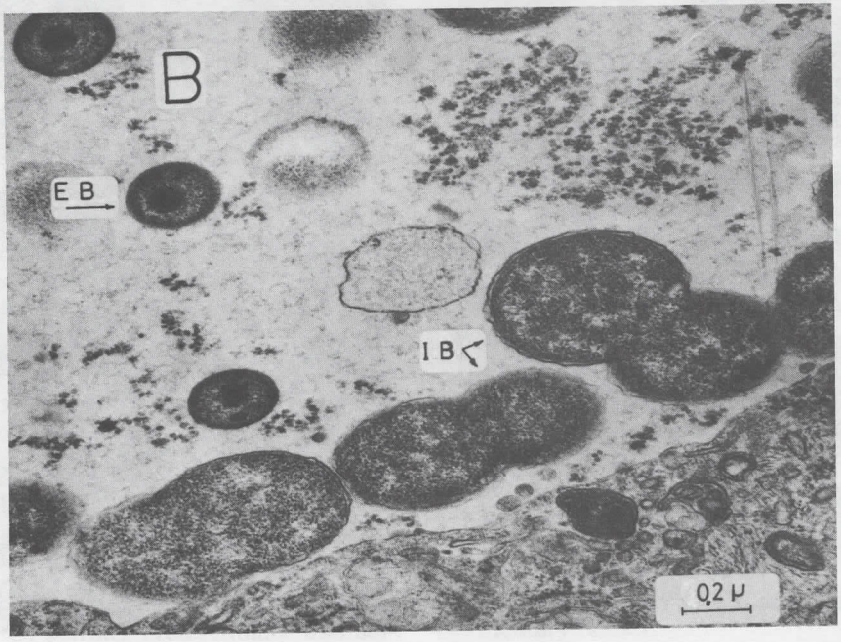


Fig. 30-7. A. Electron micrograph of *Chlamydia trachomatis* inclusion body in the cytoplasm (*C*) of an infected cell. Part of the nucleus (*N*) and mitochondria (*M*) can also be seen. B. Enlarged view of rectangle in A, showing the elementary body (*EB*) and reticulate or initial bodies (*IB*). (Courtesy Y. Becker.)





sion body and the host cell to infect adjacent cells.

The mechanisms by which chlamydiae affect host cell activity are not totally understood. Experimental results indicate the number of infectious chlamydial particles infecting the cell (multiplicity of infection, or MOI) affects the outcome. At low multiplicities of infection the host cell may divide with one daughter cell free of infection and the other killed by the infectious process. At higher MOI's host cells die in the absence of chlamydial replication. A heat-labile toxin is believed to be responsible for the pathogenesis of chlamydiae.

Chlamydial infections are divided into two groups: those in which there is person-to-person transmission (*C. trachomatis*) and those resulting from accidental contact with birds (*C. psittaci*).

Chlamydia trachomatis

OCULAR DISEASE. *Trachoma* is an ocular disease that is worldwide in distribution but is endemic in certain areas such as the Punjab region of India, isolated regions of Africa, and a few south central states in the United States. The microorganism is transmitted from person to person by fingers or fomites. Trachoma is a chronic conjunctivitis in which there is an accumulation and coalescence of lymphocytes and macrophages that form discrete red or yellow follicles on the conjunctiva. The follicles eventually enlarge and involve greater areas of the conjunctiva. Necrosis of the follicles may result in conjunctival scarring. Over a period of years these scars contract and turn in the upper eyelid, resulting in abrasion of the cornea by eyelashes. Blindness may result from mechanical scarring and secondary bacterial pathogens (*Hemophilus* or *Moraxella* species).

Inclusion conjunctivitis is a disease that may affect infants and adults. In the infant transmission occurs as a result of contact with the female genital tract during passage through the birth canal. Adult disease occurs from contact with microorganisms derived from the genital tract. Many cases of adult ocular disease are the result of swimming in pools contaminated with the microorganisms. In both infants and adults inclusion conjunctivitis is usually considered a self-limiting disease; however, scarring has been noted in infants in whom treatment has been delayed. Visual loss is infrequently observed in both groups.

Neonatal pneumonia caused by *C. trachomatis* is a frequent complication after conjunctivitis, but it may also occur in the absence of eye infection. The disease appears 1–3 months after birth and is accompanied by symptoms of spasmodic coughing. Approximately 10 to 20 percent of the infants infected with chlamydiae will develop pneumonia.

MALE GENITAL TRACT DISEASE. In industrialized countries chlamydial transmission is usually by sexual means. Chlamydiae are not part of the normal flora of the male or female genital tract. *C. trachomatis* is the causal agent of over 50 percent of the cases of *nongonococcal urethritis* (NGU)—*Ureaplasma urealyticum* is the other causal agent of this disease (see Chapter 29). NGU appears primarily in sexually active males between 16 and 30 years of age. In addition many men who have been successfully treated with penicillin for gonococcal disease demonstrate a urethritis indistinguishable from NGU called *postgonococcal urethritis*. *C. trachomatis* as well as mycoplasmas have been implicated as causal agents in this disease.

C. trachomatis has been isolated from the urethras of nearly 50 percent of men with NGU and 25 to 30 percent of those with gonorrhea. The highest rate of *C. trachomatis* among men was discovered in those who were promiscuous or who had recently changed sexual partners. Most of the female sex partners of men with chlamydial infection also carried *C. trachomatis* on the cervix. *C. trachomatis* is seldom isolated from the female sex partner of men without NGU. NGU is considered self-limiting, although a small proportion of men incur epididymitis. It takes 2–3 weeks for the manifestation of NGU, that is, a white urethral discharge, to occur (the incubation period for gonorrhea is 2–7 days).

FEMALE GENITAL TRACT DISEASE. *C. trachomatis* is the most frequent sexually transmitted pathogen in

Fig. 30-8. Lymphogranuloma venereum. Acute buboe, which has been surgically incised. (From E. V. Hamm. In W. A. D. Anderson and J. M. Kissane [Eds.], *Pathology* [7th ed.]. St. Louis: Mosby, 1977.)

the United States. The organism is a common cause of disease of the endocervix. The rate of carriage of *C. trachomatis* in pregnant women is between 2 and 18 percent. These infected pregnant women transmit the organism to their newborns, causing neonatal conjunctivitis and pneumonia. Postpartum *salpingitis* (inflammation of the fallopian tubes) is associated with chlamydial infection of the cervix. Chlamydial infection in women is also an important cause of pelvic inflammatory disease (PID), which can result in infertility, stillbirth, or premature birth.

LYMPHOGRANULOMA VENEREUM. Lymphogranuloma venereum (LGV) is a sexually transmitted disease caused by *C. trachomatis*. Fewer than 400 cases are reported each year in the United States, and the majority of these are from the southern states. A lesion develops at the site of inoculation after an incubation period of 1–4 weeks (Fig. 30-8). This is followed by lymphatic involvement and enlarged inguinal lymph nodes. As the disease progresses more lymph nodes become involved and exhibit a purplish cast. Lymph nodes may become necrotic, but they eventually heal. The most severe complications of this disease are associated with perianal abscesses and fistulas that may develop 1–10 years after infection.

Chlamydia psittaci

C. psittaci is the causal agent of psittacosis. *C. psittaci* infections occur in avian and mammalian populations, but only the avian strains are known to cause disease in humans. Psittacosis is primarily an occupational disease of those working in poultry-processing plants. Pet birds and occasionally wild birds transmit the infectious agent to humans. Infected birds usually show no clinical signs of disease. (See boxed essay, p. 635.)

Psittacosis in humans is usually asymptomatic, but an atypical pneumonia may develop. The incubation period of 1–3 weeks is followed by chills, fever, headache, and persistent cough. The symptoms subside in less than 2 weeks.

LABORATORY DIAGNOSIS

Recent advances in chlamydial isolation procedures have made it technically feasible for clinical microbiology laboratories to isolate *C. trachomatis* as a routine procedure. Cell culture systems are now commercially available. Intracytoplasmic inclusions can be identified in infected cell cultures by staining (Giemsa, Macchiavello, Gimenez, or iodine stains). Fluorescent antibody can also be used to identify the organism in cell culture smears. *C. trachomatis* and *C. psittaci* can be differentiated on the basis of sulfonamide sensitivity and iodine staining. *C. trachomatis* strains are sensitive to sulfonamides and produce an iodine-staining glycogen-like material in the inclusion body. *C. psittaci* is resistant to sulfonamides, and its inclusions do not stain with iodine. Direct microscopical exami-

PSITTACOSIS ASSOCIATED WITH TURKEY PROCESSING—OHIO

An outbreak of psittacosis occurred among employees of an Ohio turkey-processing plant in July 1981. Approximately 27 of the plant's some 80 employees were ill; 3 were hospitalized. Turkeys being slaughtered at the plant were the probable source of infection, but no specific group of birds could be implicated.

Most patients had an illness characterized by weakness, headache, fever, chills, and cough. To a lesser extent, patients had photophobia, conjunctival suffusion, generalized joint pains, stomach cramps, and diarrhea. Eight patients who had chest x-rays showed evidence of pneumonia consistent with psittacosis.

Paired serum specimens from 27 workers were tested for complement-fixing antibodies to chlamydial group antigen. Of 15 workers who had recently had an illness compatible with psittacosis, 7 had a $\geq$ fourfold titer rise, and 5 had a titer of $\geq$ 16 in at least one specimen. Of 12 workers who had not recently had a compatible illness, none had a significant titer change. Single serum specimens were obtained from 29 other workers 1–3 days after onset of the last recognized case in the employee group. Eight of 11 workers in this group who had recently had illness compatible with psittacosis had a titer of $\geq$ 16; 2 of 18 who had not had such an illness had a titer $\geq$ 16.

The plant, which operates approximately 40 hours per week, 10 months a year, processes turkeys only, which are delivered by truck from various locations, slaughtered, and defeathered on the day of arrival in the "kill-pick" area. Then they are conveyed on a continuously moving line into the evisceration area where deep tissues are exposed, the birds are inspected and trimmed, edible organs are removed, and the remaining inedible internal and external parts are discarded.

Because most employees worked in various job stations in several departments on a given day, it was difficult to assess the relative importance of respiratory, skin, and conjunctival exposure. However, the attack rate by work department was significantly higher for workers in the kill-pick and evisceration areas than in other departments of the plant. Futhermore, there was no apparent correlation between degree of skin exposure and clinical psittacosis, which suggests that infections were the result of aerosol transmission or that multiple routes of exposure may have been involved.

Turkey condemnation rates were analyzed in an effort to identify a specific flock or flocks that were the source of infection. Investigations by public health veterinarians in the states of origin for these birds revealed no evidence of psittacosis at the implicated sites.

From *MMWR* 30:638, 1982.

nation of clinical isolates is often insensitive and difficult. Microscopical methods are seldom if ever used in the diagnosis of LGV or psittacosis.

The complement fixation test is the most widely used serological test for diagnosing chlamydial disease. It is useful for diagnosing psittacosis and LGV but less useful in diagnosing oculogenital infections and nearly useless in diagnosing trachoma. A more sensitive serological test is the microimmunofluorescence (MIF) test, which is useful in diagnosing oculogenital infections and trachoma. A commercial antigen, however, is not available for the MIF test, and each laboratory must prepare the antigen by growing *Chlamydia* in yolk sacs.

A simple half-hour slide test using monoclonal antibodies is now available for diagnosis of chlamydial venereal disease. The urethra or cervix is swabbed, and the sample is smeared on a microscopic slide. Monoclonal antibodies stained with fluorescent dye are doused on the slide, which is washed and then observed microscopically for fluorescence.

TREATMENT AND PREVENTION

Tetracyclines are the drug of choice in the treatment of most chlamydial disease; erythromycin is sometimes an alternative. Sulfisoxazole is a useful alternative to tetracycline in the treatment of those who are pregnant. Erythromycin syrup administered orally is used in the treatment of established inclusion conjunctivitis in the newborn as well as infant pneumonia. For uncomplicated infection in adults tetracycline is the recommended drug, but alternatives include amoxicillin or ampicillin or aqueous penicillin G procaine.

Vaccines have been used on a trial basis for preventing trachoma, but they have so far demonstrated little success. Prevention of psittacosis is dependent on preventing avian infection by chemoprophylaxis. Imported birds are supposed to be treated at quarantine stations; however, these measures are oftentimes ignored or improperly carried out.

Silver nitrate drops, which were originally used on newborns to prevent gonococcal infection of the eye, have been replaced by antibiotics such as erythromycin or tetracycline. These agents can prevent chlamydial conjunctivitis.

SUMMARY

Rickettsiae are gram-negative intracellular parasites that cannot be cultivated on synthetic laboratory media. Except for *Coxiella burnetii*, the rickettsiae are transmitted to humans by arthropod vectors. Rickettsial infections are characterized by the affinity of the microorganisms for vascular tissue. A necrotic lesion often appears at the site of the arthropod bite. The most common rickettsial diseases in the United States are Rocky Mountain spotted fever and Q fever. Rocky Mountain spotted fever is transmitted to humans by ticks that are harbored on rodents and various wild animals. Untreated, the disease is often fatal. Tetracycline is the drug of choice in treatment. Q fever is primarily an occupational disease for those working with dairy cattle and other livestock. Disease is acquired by direct or indirect contact with contaminated animals or animal products. The disease is usually mild, but in those with heart disease endocarditis can be a sequela of Q fever. The remaining rickettsial diseases include various types of typhus, trench fever, and rickettsialpox. Most rickettsial diseases are best diagnosed by serological tests such as complement fixation.

The chlamydiae are gram-negative intracellular parasites that are characterized by their unique developmental cycle, which alternates between two forms, the infectious elementary body and the noninfectious reticulate body. Two species of *Chlamydia, C. trachomatis* and *C. psittaci*, are human pathogens. *C. trachomatis* is the causal agent of trachoma and inclusion conjunctivitis, both ocular diseases, as well as male and female genital

tract diseases such as nongonococcal urethritis in males and salpingitis in females. Lymphogranuloma venereum, another sexually transmitted disease caused by *C. trachomatis*, affects both males and females. *C. psittaci* affects avian populations but is secondarily transmitted to humans (psittacosis). Tetracyclines are used in the treatment of chlamydial disease.

QUESTIONS FOR STUDY

1. What are the most common rickettsial diseases in the United States?
2. What is the major metabolic difference between the rickettsiae and chlamydiae?
3. What group of people are most likely to get Rocky Mountain spotted fever?
4. What might be the explanation for the ability of the agent of Q fever to survive outside the animal host while all other rickettsiae cannot?
5. Describe the developmental cycle of the chlamydiae during the infectious disease process.
6. What is meant by *nongonococcal urethritis* and *postgonococcal urethritis*? What are the causal agents of these diseases?
7. Why is carriage of chlamydiae in the female genital tract potentially dangerous?
8. What antibiotics are effective in the treatment of rickettsial and chlamydial diseases?
9. What is one virulence factor of the rickettsiae?
10. What is the reservoir of *C. psittaci*?

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31. ACTINOMYCETES

OBJECTIVES

To list the most important species of *Actinomyces* and the types of infections with which they are associated

To outline the similarities and differences between *Nocardia* and *Actinomyces*

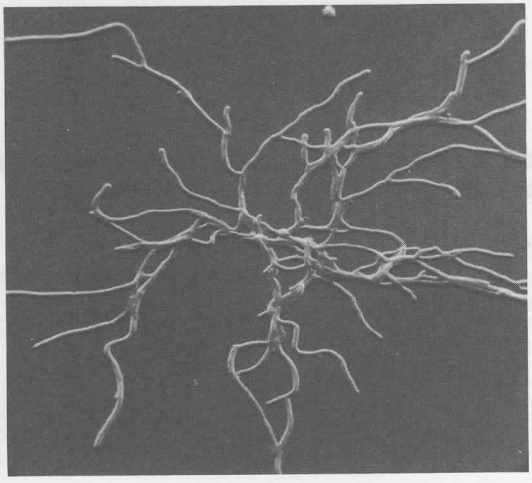
To describe the types of infections associated with *Nocardia*

To describe the types of treatment required for actinomycoses and nocardioses

Genus *Actinomyces*
General Characteristics
Pathogenesis
Actinomycosis
Periodontal Disease
Laboratory Diagnosis
Treatment
Genus *Nocardia*
General Characteristics
Pathogenesis
Laboratory Diagnosis
Treatment

Actinomycetes refers to a large group of filamentous bacteria that are found primarily in the soil but also include genera indigenous to animals and humans. Some of the pathogens indigenous to humans are species of *Corynebacterium* and *Mycobacterium*, which were discussed previously. *Streptomyces*, an important actinomycete found in the soil, is composed of species responsible for the production of many currently used antibiotics such as chloramphenicol, tetracycline, erythromycin, streptomycin, amphotericin B, and others. Two genera of actinomycetes that are human pathogens are *Actinomyces* and *Nocardia*. One of the distinguishing characteristics of *Actinomyces* and *Nocardia* is their ability to produce branching filaments, as do the fungi. Unlike the fungi, the actinomycetes are prokaryotic cells that are gram-positive and possess a peptidoglycan layer. Thus actinomycetes are susceptible to penicillin, a further characteristic that distinguishes them from fungi.

Fig. 31-1. Scanning electron micrograph of *Actinomyces israelii* microcolony. Note the branching filaments. (From J. M. Slack and M. A. Gerencser, *Actinomyces, Filamentous Bacteria: Biology and Pathogenicity*. Minneapolis: Burgess, 1975.)



GENUS *ACTINOMYCES*

GENERAL CHARACTERISTICS

Species of the genus *Actinomyces* that are pathogenic for animals and humans are *A. bovis, A. israelii, A. odontolyticus, A. naeslundii*, and *A. viscosus*. Except for *A. israelii* all of these species are facultative anaerobes. They all produce branching filaments during growth (Fig. 31-1), but no aerial filaments are produced by this genus. All species make up part of the microbial flora of the oral cavity and female genital tract. Diseases caused by *Actinomyces* are called *actinomycoses*.

PATHOGENESIS

Actinomycosis

Actinomycosis is a chronic, granulomatous disease characterized by abscess formation and draining sinuses. *A. bovis* is the causal agent of actinomycosis in cattle, called "lumpy jaw," which is similar

Fig. 31-2. *Actinomyces* sulfur granule in tissue. (Courtesy K. J. Kwon-Chung.)

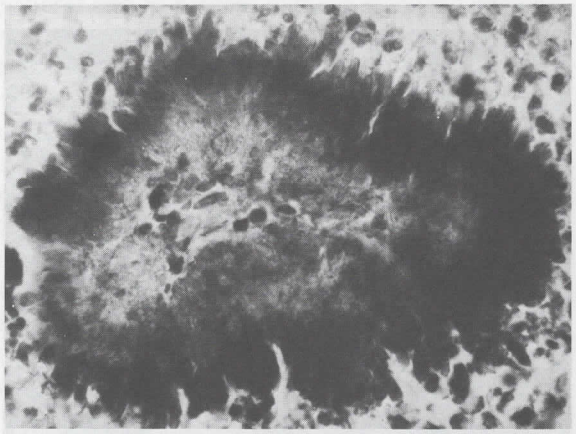


Fig. 31-3. Cervicofacial actinomycosis. (Courtesy A. Gonzalez-Ochoa.)

to the human variety caused by *A. israelii* and *A. naeslundii*. The draining sinuses produced during disease contain white or yellow granules composed of microcolonies of the branching actinomycete (Fig. 31-2). Four types of actinomycosis are now recognized: cervicofacial, thoracic, abdominal, and genital.

Cervicofacial actinomycosis is the most common type of actinomycosis and is usually associated with the lower jaw. Individuals predisposed by conditions such as tooth decay, jaw fracture, or tooth extraction are most susceptible to actinomycosis. Disease is characterized by abscess formation and development of sinus tracts (Fig. 31-3) that eventually reach the skin, producing nodules that can break and form fistulas. The tissue around the fistulas often shrinks, leaving an area of depression. In advanced cases the infection may penetrate the orbital cavity causing blindness.

Thoracic actinomycosis can result from the aspiration of microorganisms from the oral cavity, by extension from cervicofacial actinomycosis, or by hematogenous spreading. Lesions occur in the lung and may involve both lobes. The infection extends to the pleura and eventually penetrates the thoracic wall through multiple draining sinuses. Fistulas and the characteristic depression on the chest are also evident. During the course of the disease there may be chest pains, fever, and cough resembling those of other respiratory diseases such as tuberculosis.

Abdominal actinomycosis results most frequently from trauma of the intestinal tract or abdominal wall. Many cases of disease are a consequence of appendectomies that fail to heal or perforated appendixes. Sinus tracts may develop and pentrate through the abdominal wall.

Genital actinomycosis is a relatively new clinical syndrome resulting from the wearing of intrauterine devices.

Periodontal Disease

A. viscosus and *A. naeslundii* are indigenous to the oral cavity and have been associated with periodontal disease. Both species produce pili (fimbriae) that are apparently required for attachment to epithelial surfaces and the aggregation of microbial species in dental plaque. The adherence of strains of *A. viscosus* that cause periodontal disease has been studied in laboratory animal models. A mutant of this pathogen produces excessive amounts

of extracellular slime that interferes with adherence to epithelial cells. Apparently the slime produced by the mutant masks the adhesins on the microbial surface and thus prevents attachment (see Chapter 37 for further discussion of periodontal disease).

LABORATORY DIAGNOSIS

Pus from fistulas and sputum can be examined for the appearance of yellow or white granules. When granules are not present in the fistulas, the wound should be covered with gauze and examined the next day. Gram-staining the granules permits the identification of gram-positive filamentous or diphtheroid bacilli. Clinical specimens or granules should be inoculated into thioglycolate broth or brain-heart infusion agar and incubated anaerobically for 2–5 days. When colonies develop, the growth should be checked for the appearance of gram-positive bacilli. Species of *Actinomyces* can be identified directly in clinical specimens by immunofluorescence techniques.

TREATMENT

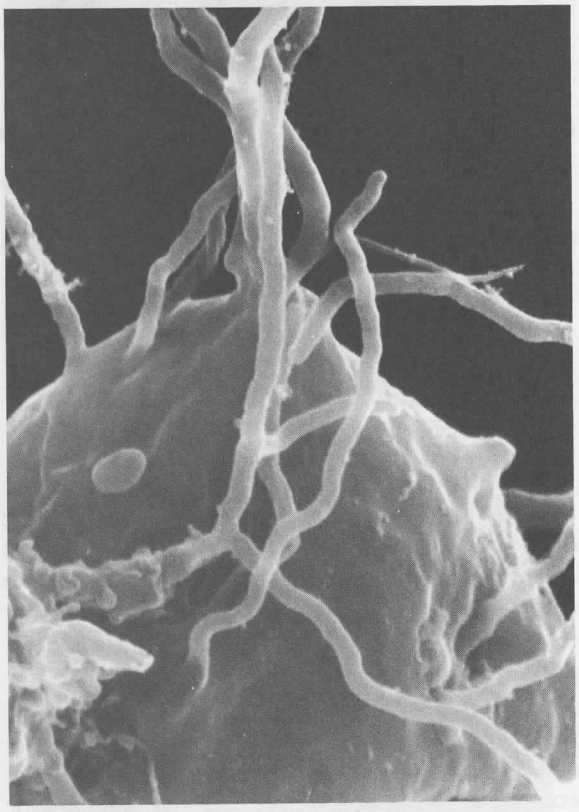
Surgical debridement of damaged tissue is the prerequisite to antibiotic therapy. Penicillin G administered over a period of 3–4 weeks remains the drug of choice. Recent evidence indicates that combinations of trimethoprim and sulfamethoxazole have resulted in more rapid cures, particularly in cervicofacial and abdominal actinomycoses.

GENUS *NOCARDIA*

GENERAL CHARACTERISTICS

All species of *Nocardia* are found in the soil. They are aerobic, gram-positive, nonsporeforming bacilli (Fig. 31-4). Unlike the genus *Actinomyces, Nocardia* produce aerial filaments that can undergo fragmentation into individual cells. In addition most pathogenic *Nocardia* often produce pigmented colonies that may be yellow, orange, red, or white, while *Actinomyces* produce buff or white colonies. *Nocardia* are noted for their ability to decompose paraffin, a property that can be used to isolate *Nocardia* selectively from soil environments. The most pathogenic species of *Nocardia* is *N. asteroides*, but *N. caviae* and *N. brasiliensis* are also involved in some disease.

Fig. 31-4. Scanning electron micrograph of rabbit alveolar macrophage infected with filamentous *Nocardia*. (From B. L. Beaman, *Infect. Immun.* 15:925, 1977.)



PATHOGENESIS

Diseases caused by *Nocardia* are called *nocardioses* and usually involve compromised patients.

The immunosuppressed and those with cancer or chronic pulmonary disease are more susceptible to infection. Approximately 15 percent of transplant patients and 53 percent of those receiving immunosuppressive therapy develop nocardiosis. Most infections result from inhalation of the organism or direct inoculation into traumatized tissue. The site of infection is the lungs, where nodular abscesses are most frequently observed. Fever and dry cough are symptoms sometimes observed, but many times no symptoms are evident. If not diagnosed and treated early, the infection may become metastatic and involve the central nervous system and kidneys. Abscess formation may occur in the brain and kidneys. Fatality rates approach 80 percent in undiagnosed disseminated disease.

N. brasiliensis is occasionally associated with nocardiosis but is primarily a cause of mycetoma. *Mycetoma* is a chronic, suppurative, granulomatous disease of the subcutaneous tissue. The disease, which usually involves the foot, is also caused by several species of fungi (see Chapter 35).

Many studies have been made of the mechanism of pathogenesis of *N. asteroides*. Virulent and nonvirulent strains of this species are phagocytized by neutrophils, but only virulent strains resist killing. The virulent strains inhibit phagosome-lysosome fusion, and even if fusion does occur the effectiveness of the degradative enzymes is diminished. Some studies have demonstrated a direct correlation between resistance and the level of catalase in the bacterial strain. The virulent strains are resistant to hydrogen peroxide, hydroxyl radical, and singlet oxygen, which are generated by oxidative burst in neutrophils.

LABORATORY DIAGNOSIS

Exudative material from lesions should be examined microscopically for microcolonies of granules. Sputum, spinal fluid, urine, and exudative material can be examined microscopically for typically thin, highly branched filaments. Acid-fast staining techniques have revealed that young colonies are strongly acid-fast, while older cultures are variable. Specimens should be planted on Sabouraud dextrose agar slants (without antibiotics) and streaked on beef heart infusion agar plates and incubated aerobically at 25–30°C and at 37°C. Cultures are examined at 48-hour intervals for 2 weeks or more. Colonies on both media initially appear yellow but on further incubation turn a dark orange. Aerial filaments can be discerned microscopically. Filaments have a tendency to break up into bacillary forms. A variety of biochemical tests can be used to differentiate *Nocardia* from other actinomycetes.

TREATMENT

As in actinomycosis, surgical debridement of damaged tissue should be performed whenever possible before chemotherapy is initiated. *N. asteroides* responds quickly to treatment. Its early diagnosis is important since recovery rates after systemic infection are very low. Sulfonamides are the drug of choice in treatment. Trimethoprim-sulfamethoxazole is highly effective but whether it is better than sulfonamide is not yet known.

SUMMARY

Actinomyces and *Nocardia* are gram-positive bacilli that produce filamentous growth. *Actinomyces* produce no aerial filaments, but *Nocardia* do produce aerial filaments. Several species of *Actinomyces* indigenous to the oral cavity produce disease in humans. Actinomycosis is a chronic granulomatous disease in which there is swelling and formation of sinus tracts. Yellow-white granules composed of the microbial filaments are found in the lesions. Actinomycosis is divided into four types: cervicofacial, thoracic, abdominal, and genital. Most disease occurs in compromised patients and is caused primarily by *A. israelii* and *A. naeslundii*. *Actinomyces viscosus* and *A. naeslundii* are associated with periodontal disease. They adhere

to epithelial cells by means of pili. Clinical specimens are examined for yellow-white granules and gram-positive filamentous microorganisms. Treatment involves surgical debridement and penicillin therapy.

Nocardia species are found in the soil and produce pigmented colonies when grown in the laboratory. They are capable of metabolizing paraffin, a characteristic that distinguishes *Nocardia* from other genera. Nocardiosis occurs primarily in compromised patients such as those receiving immunosuppressive therapy. The lung is the site of infection, but metastasis to other organs occurs if the disease is not treated early. *N. asteroides* is the primary cause of lung infection, while *N. brasiliensis* is one cause of mycetoma, a subcutaneous disease affecting primarily the foot. *Nocardia* can be diagnosed by cultural examination and biochemical tests. Sulfonamides are the drugs of choice in treatment of disease.

QUESTIONS FOR STUDY

1. What is characteristic of the lesions in actinomycosis?
2. What species are associated with actinomycosis?
3. What metabolic characteristic of *Nocardia* distinguishes it from most other genera of microorganisms?
4. What morphological characteristic distinguishes *Nocardia* from *Actinomyces*?
5. What is the normal habitat of *Actinomyces* species? *Nocardia* species?
6. What is the mechanism of virulence for *N. asteroides*?

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32. MISCELLANEOUS PATHOGENS

OBJECTIVES

To describe the method of transmission, sources of disease, site of infection, and treatment for Legionnaires' disease

To list the type of infection associated with *Capnocytophaga* species, *Actinobacillus*, *Calymmatobacterium*, and *Streptobacillus*

Legionella
General Characteristics
Epidemiology
Pathogenesis
Laboratory Diagnosis
Treatment

There are some unclassified microorganisms pathogenic for humans that have not been discussed. They include genera such as *Streptobacillus, Actinobacillus, Capnocytophaga, Calymmatobacterium, Legionella, Cardiobacterium*, and *Chromobacterium*. We will give most of our attention to *Legionella* because it is a recently discovered pathogen of clinical importance. The remaining genera are outlined in Table 32-1.

LEGIONELLA

More than 200 persons were stricken with a respiratory disease of unknown origin at the American Legion Convention in Philadelphia in 1976, and 34 died. The causal agent of the disease at first resisted isolation, but researchers at the Centers for Disease Control in Atlanta identified the previously unknown bacterium and called it the *legionnaires' disease bacterium* (LDB), now classified as *Legionella pneumophila*. The genus *Legionella* belongs to the family Legionellaceae.

GENERAL CHARACTERISTICS

The causal agent of legionnaires' disease is a gram-negative aerobic bacillus 0.3 to 0.4 μm in diameter and 2 to 3 μm in length (Fig. 32-1). Occasionally the organism appears filamentous, reaching up to 20 μm in length. The organism does not grow on the usual bacteriological media. Clinical specimens from lung tissue taken at autopsy were originally cultivated in the yolk sac of embryonated eggs. Recently, supplemented laboratory media have been used successfully to cultivate the bacillus.

Taxonomy of the Legionellaceae is in a state of flux. There are at least 12 published species, some of which have not been recovered from human infections. Those identified as human pathogens present clinical, epidemiological, and bacteriological features similar to those of *L. pneumophila*. Some of the isolated species are described in Table 32-2.

EPIDEMIOLOGY

L. pneumophila is an organism found primarily in water. It has been found in thermal effluent water, creek water, mud, water from cooling towers, and water from freshwater lakes. *L. pneumophila* is not thermophilic but can survive in water at temperatures from 6–67°C. Outbreaks of disease have been associated with heat-exchange apparatuses, shower water, tap water, and potable water after chlorination. The most important mechanism of transmission is via aerosols.

L. pneumophila may be acquired in the community or in the hospital. In about 70 percent of the cases the patient has had some underlying condition or illness that predisposed him or her to disease by *Legionella*. Surveys of several healthy populations have demonstrated a high titer of antibody to *L. pneumophila*. This may indicate that the organism is abundant in the environment and is responsible for asymptomatic infection in the healthy population. Predisposing factors contributing to disease are diabetes, cancer, immunosuppression, smoking, renal dialysis or transplantation, construction work as an occupation, and alcohol abuse.

Table 32-1. Characteristics of Miscellaneous Bacterial Species Pathogenic to Humans

Species	Characteristics of disease	Characteristics of organism	Treatment
Calymmatobacterium granulomatis	Granuloma inguinale, an infrequent sexually transmitted disease: lesions in pubic area that may lead to elephantiasis; occasionally found in southern United States	Pleomorphic, gram-negative, nonmotile rod that is heavily encapsulated	Streptomycin, tetracycline, or erythromycin
Streptobacillus moniliformis	One type of rat-bite fever acquired by bite of rat or ingestion of contaminated food; fever, rash, and polyarthritis are major symptoms	Pleomorphic, facultatively anaerobic gram-negative rod; normal inhabitant of rat throat and nasopharynx	Penicillin or streptomycin; penicillin induces L-forms
Actinobacillus actinomycetemcomitans	Found associated with *Actinomyces* species in disease	Nonmotile, gram-negative, facultatively anaerobic coccobacillus; part of human oral flora	Tetracyclines, chloramphenicol, trimethoprim-sulfamethoxazole
Capnocytophaga species	Juvenile periodontosis and bacteremia in immunosuppressed	Gram-negative gliding bacterium; normal inhabitant of human oral cavity; *C. ochracea* most important pathogen	Penicillin
Cardiobacterium hominis	Recovered in cases of endocarditis	Pleomorphic, gram-negative, nonmotile rod; normal inhabitant of respiratory and intestinal tract of humans	Penicillin, tetracyclines, aminoglycosides
Chromobacterium violaceum	Abscess formation and bacteremia; human infections often fatal; septicemia invariably fatal; most cases in Southeast Asia and southeastern United States	Motile, gram-negative, slightly curved rod; found in soil and water; produces violet pigment in broth and on agar	Aminoglycosides, chloramphenicol, tetracyclines

Fig. 32-1. Photomicrograph of *Legionella pneumophila*. Bacterial cells are observed as plump rods (*arrow*). (Courtesy Centers for Disease Control.)

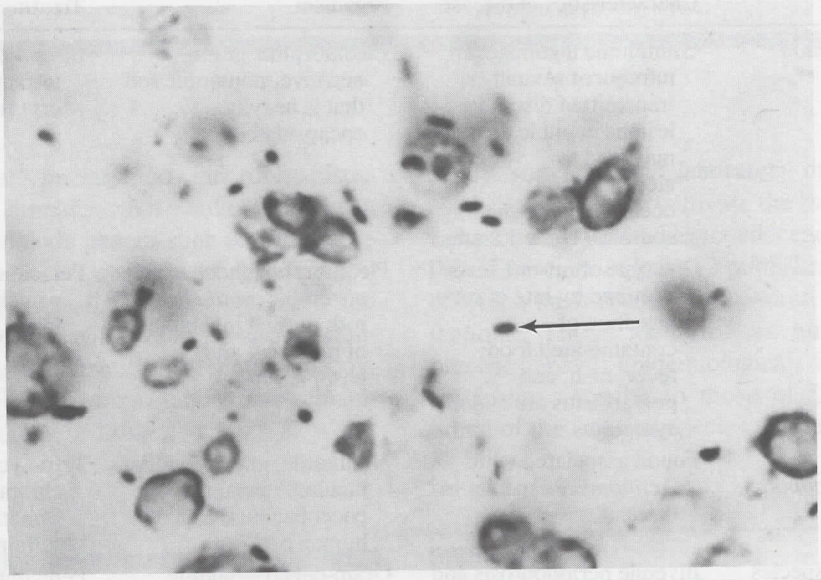


Table 32-2. Some of the Isolated Species of *Legionella*

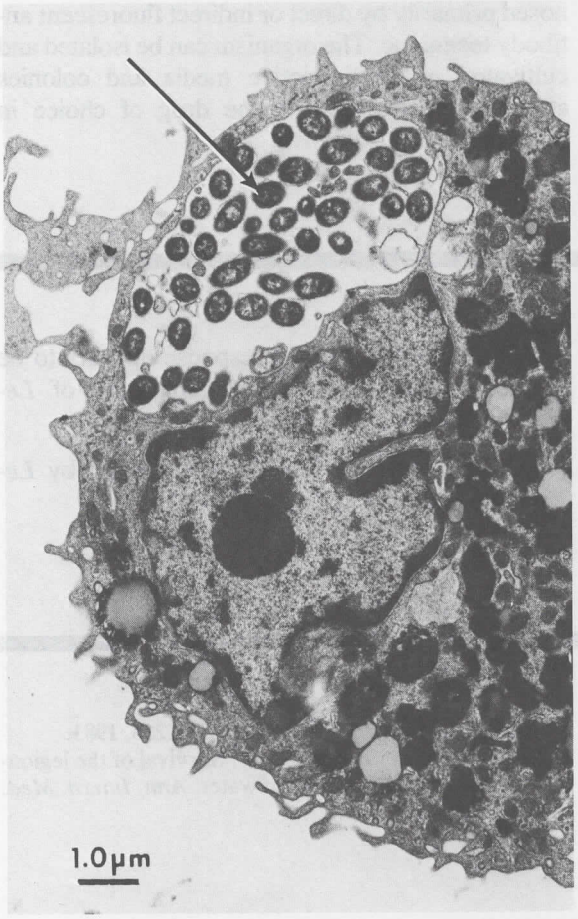
Species	Original source of isolation
L. pneumophila	Human respiratory tissue
L. bozemanii	Human respiratory tissue
L. dumoffii	Human respiratory tissue
L. micdadei	Enzootic in pigs
L. gormanii	Creek bank
L. jordanis	Water and sewage
L. longbeachae	Human respiratory tissue

PATHOGENESIS

Few experimental animal model studies of the pathogenesis of *L. pneumophila* have been done. The microorganism is a facultative intracellular parasite that multiplies within the mononuclear phagocyte but not in neutrophils or lymphocytes (Fig. 32-2). Within the monocyte the bacteria are surrounded by a membrane-bound cytoplasmic vacuole that is studded with ribosome-like particles. Neutrophils and monocytes phagocytize *L. pneumophila* only in the presence of complement and antibody. They do not destroy all the bacilli, and surviving bacilli are capable of multiplication in the monocyte. Humoral immune responses are apparently not effective in defense against the organism; the developers of vaccines will have to take this into account. A variety of enzymes and products are released by *L. pneumophila*, but their role, if any, in pathogenesis is not known.

Disease caused by *L. pneumophila* is respiratory and may be mild, requiring no hospitalization, or it may be severe pneumonia requiring hospitalization. The incubation period is 2–10 days. The onset of disease is usually gradual except in severely compromised patients such as the immunosuppressed. The first symptoms are fever, headache, and weakness, followed by sore throat and rhinitis. Diarrhea may appear before or after respiratory symptoms. The fever is unremitting, and in about one-third of the cases there is lethargy, confusion,

Fig. 32-2. Electron micrograph (×8000) of *Legionella pneumophila*. Several cells (*arrow*) are observed in a distended vesicle in an alveolar macrophage. (From R. A. Kishimoto, et al., *Infect. Immun.* 25:761, 1979.)



disorientation, and other central nervous system symptoms. The major pathological findings are confined to respiratory tissue. Severe bronchopneumonia with inflammation in the alveoli and respiratory bronchioles is common. Extrapulmonary manifestations include central nervous system symptoms such as tremors of the extremities and hyperactive reflexes. Most extrapulmonary manifestations are found in immunosuppressed patients.

LABORATORY DIAGNOSIS

Three methods are currently used for the diagnosis of legionnaires' disease. The most widely used is the indirect fluorescent antibody (IFA) technique. An increase in IFA titer of at least fourfold (1:128) in serum specimens obtained during the acute and convalescent phases of disease is considered confirmatory. Only 75 percent of patients with clinical disease develop this fourfold rise in titer early in the disease.

Direct fluorescent antibody (DFA) examination of respiratory secretions, lung tissue, or other tissue specimens is the most rapid means of diagnosis. A high level of skill is required to perform the tests, and many of the materials required for the test are not readily available in some areas.

The third technique involves the cultivation and isolation of *Legionella* species on laboratory media. Semiselective media are required for isolation from contaminated specimens, but for specimens free from other bacteria nonselective media, such as charcoal-yeast extract, are suitable. Colonies appear 3–8 days after inoculation and can be stained using special stains such as silver plating stain to identify individual cells. A definitive diagnosis can be made only by direct fluorescent stains, gas-liquid chromatography, or DNA hybridization.

TREATMENT

Oral administration of erythromycin results in rapid effects within 12–36 hours. Studies have demonstrated that erythromycin reduces the case fatality ratio about fourfold compared to those who do not receive antibiotic therapy. In immunosuppressed patients without therapy the disease is invariably fatal.

SUMMARY

Legionella is the most important of the unclassified pathogens and is now divided into at least 12 species. *Legionella pneumophila*, the causal agent of

legionnaires' disease, is the prototype of the genus. It is a gram-negative aerobic bacillus that cannot be cultivated on ordinary laboratory media and must have various supplements to grow. *Legionella* species are found in water and are believed to be transmitted to humans by aerosols. Most severe cases occur among those in the hospital where underlying illness makes them more susceptible to infection.

Legionella is an intracellular parasite that multiplies in the mononuclear phagocytes of the host. Pneumonia is the most predominant clinical manifestation of disease; extrapulmonary manifestations are more evident in those patients undergoing immunosuppression therapy. The disease is diagnosed primarily by direct or indirect fluorescent antibody technique. The organism can be isolated and cultivated on semiselective media and colonies stained. Erythromycin is the drug of choice in treatment.

QUESTIONS FOR STUDY

1. Why do you think legionnaires' disease is so difficult to cure in many individuals?
2. Where are species of *Legionella* usually found?
3. What are the conditions that predispose a person to legionnaires' disease?
4. Which type of immune response appears to be effective in halting the multiplication of *Legionella* species?
5. Which host tissue is primarily affected by *Legionella* species?

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VI. VIROLOGY

33. VIRUSES

OBJECTIVES

To describe the chemical composition and structure of viruses
To explain how bacterial and animal viruses infect their hosts
To compare and contrast the infection process by temperate and lytic viruses
To differentiate between generalized and restricted transduction
To compare the multiplication cycle of RNA and DNA viruses
To explain the different techniques for cultivating viruses
To describe the effects of viral infection on the cell
To explain the techniques used to measure animal virus
To describe some of the most important laboratory procedures for the diagnosis of viral disease
To list some of the principal antiviral drugs used as chemotherapeutic agents
To list those viruses believed to be associated with cancer
To understand the current theories of how viruses may cause cancer

- Bacterial Viruses
  - Composition and Structure
  - Physiology of Virus Infection
    - Lytic Bacteriophage Infection
      - Adsorption and Injection
      - Maturation
      - Release
    - Temperate Bacteriophage Infection (Lysogeny)
  - Bacteriophages and Transduction
    - Generalized Transduction
    - Restricted Transduction
- Animal Viruses
  - Structure and Composition
    - Morphology
    - Nucleic Acids
    - Lipids
    - Proteins
  - Physiology of Animal Virus Infection
    - Adsorption
    - Penetration and Uncoating
    - Maturation
      - Replication of DNA Viruses
      - Replication of RNA Viruses
    - Release
  - Cultivation of Viruses
  - Epidemiology
  - Effects of Viral Infection on the Host Cell
    - Cytopathic Effects
    - Inclusion Body Formation
    - Cell Fusion
    - Changes in Surface Antigens
    - Interferon Production
    - Persistent Viral Infections
    - Transformation
  - Measurement of Animal Viruses
    - Infectious Virus
      - Plaque Assay
      - Pock Formation
      - Focus Formation
      - Serial Dilution End Point
    - Total Virus
      - Direct Measurement
      - Indirect Measurement
- Diagnostic Procedures in Viral Identification
  - Immunoperoxidase Test
  - Radioimmunoassay
  - Immunoelectron Microscopy
  - Enzyme-linked Immunosorbent Assay
  - Neutralization Test
  - Hemagglutination Inhibition Test
  - Gel Diffusion
  - Complement Fixation
  - Immunofluorescence
- Effect of Chemical and Physical Agents on Viral Activity
  - In Vitro Inactivating Agents
    - Agents That React with Viral Proteins
    - Agents That React with Viral Nucleic Acids
    - Agents That React with Viral Lipids
  - Antiviral Chemotherapy
    - Amantadine Hydrochloride
    - Methisazone
    - Rifampin
    - Ribonucleosides and Deoxyribonucleosides
    - Acyclovir
    - Interferon Production
  - Vaccines
- Oncogenic Viruses and Cancer
  - Properties of Transformed Cells
  - Oncogenic DNA Viruses
    - Polyomavirus and Simian Virus 40
    - BK and JC Viruses
    - Herpesviruses
      - Epstein-Barr Virus
      - Herpes Simplex Viruses
      - Marek's Disease Herpesvirus
  - Oncogenic RNA Viruses
    - Oncovirus B
    - Oncovirus C
  - Cellular Oncogenes and Carcinogenesis
- Viral Classification

Until the latter half of the nineteenth century, it was believed that certain mammalian infectious agents, capable of passing through porcelain bacterial filters and microscopically unobservable, were merely small forms of bacteria. The fact that these so-called small bacteria could not be cultivated on any laboratory medium only implied that they were fastidious in their requirements and that their needed nutritional components had not been discovered. After the turn of the century some scientists proposed that these submicroscopic agents were a subcellular form of life and called them *viruses*. About 1915 it was discovered that even bacteria were subject to disease and that the infectious agent resembled in many ways the mammalian virus. The bacterial invaders were called *bacteriophages* or simply *phages*. Not until the advent of the electron microscope and other technological advances were many of the hypotheses concerning these viruses either substantiated or discarded. In the past 40 years a plethora of information concerning viral structure and physiology has been forthcoming. A virus is now defined as a subcellular agent, consisting of a core of nucleic acid surrounded by a protein coat, that must use the metabolic machinery of a living host to replicate and produce more viral particles.

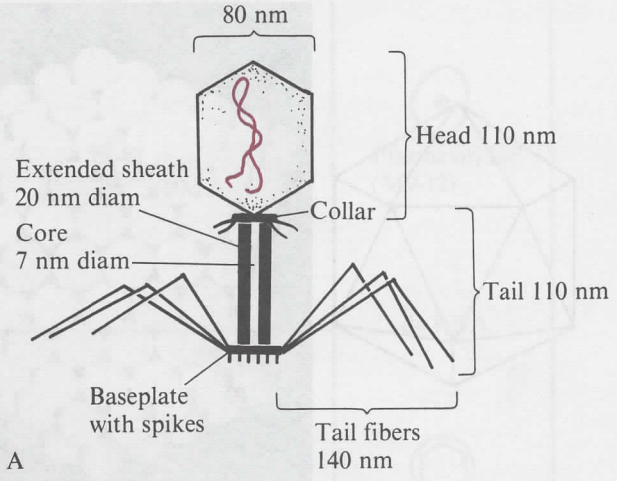
Because of their ease of cultivation, bacterial viruses have received the widest attention in viral research. Experiments exploring the metabolism and genetics of phage-infected cells have been of monumental importance to our understanding of animal viruses as infectious agents. For this reason our discussion begins with the bacterial viruses.

BACTERIAL VIRUSES

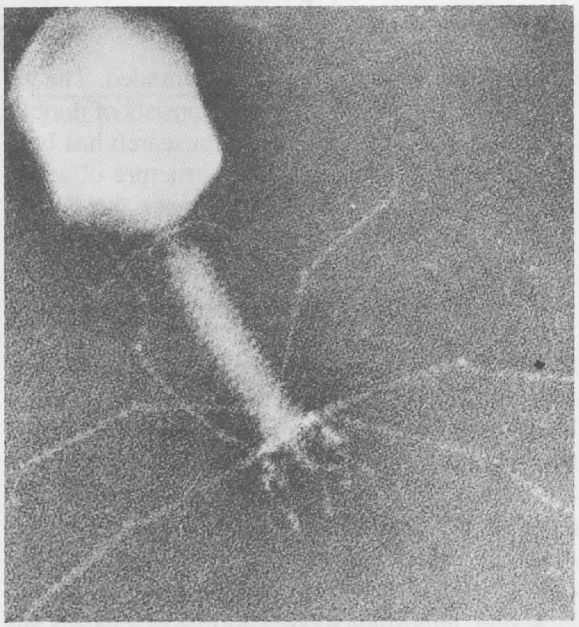
COMPOSITION AND STRUCTURE

In terms of chemical composition, the bacterial virus, like other viruses, is not a complex entity. It consists of a core of nucleic acid surrounded by a protein coat. Depending on the virus, the nucleic acid may be either DNA or RNA, and both can be

Fig. 33-1. The T4 bacteriophage. A. Diagram. B. Electron micrograph. (From R. C. Williams and H. W. Fisher, *An Electron Micrograph Atlas of Viruses*, 1974. Courtesy of Charles C Thomas, Publisher, Springfield, Illinois.)

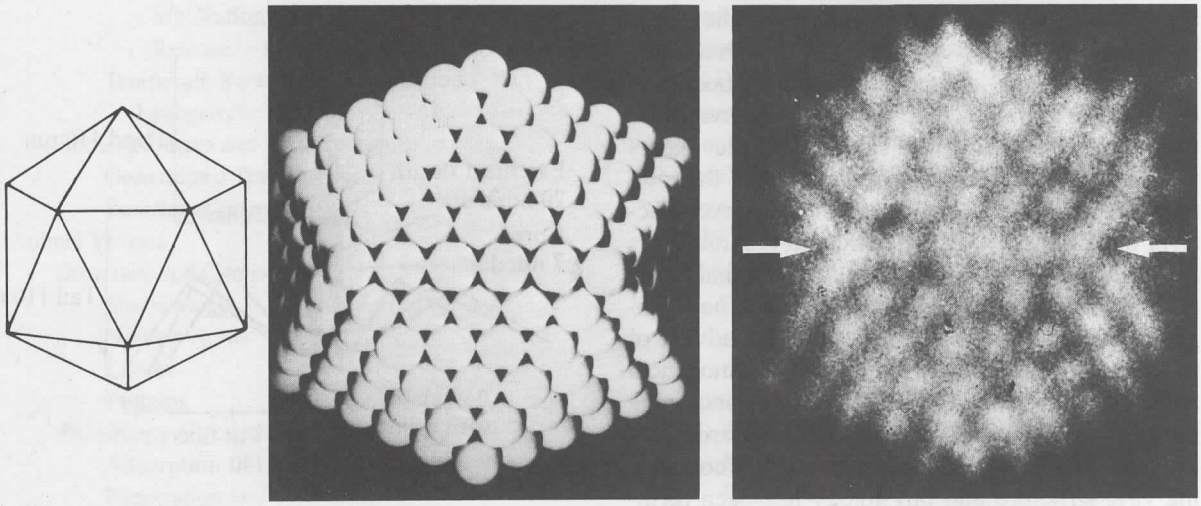


A



B

Fig. 33-2. Viral capsid symmetry. A. Icosahedral (20-sided) symmetry found in bacterial virus head protein and animal virus coat protein. B. Model of an icosahedron constructed from 252 table-tennis balls photographed in the same orientation as the particle in C. C. Negatively stained adenovirus particle showing the arrangement of capsomeres packed in accordance with 5, 3, 2 symmetry. The arrows indicate the axes of five-fold rotational symmetry. (From R. W. Horne and P. Wildy, *Br. Med. Bull.* 18:199, 1962.)



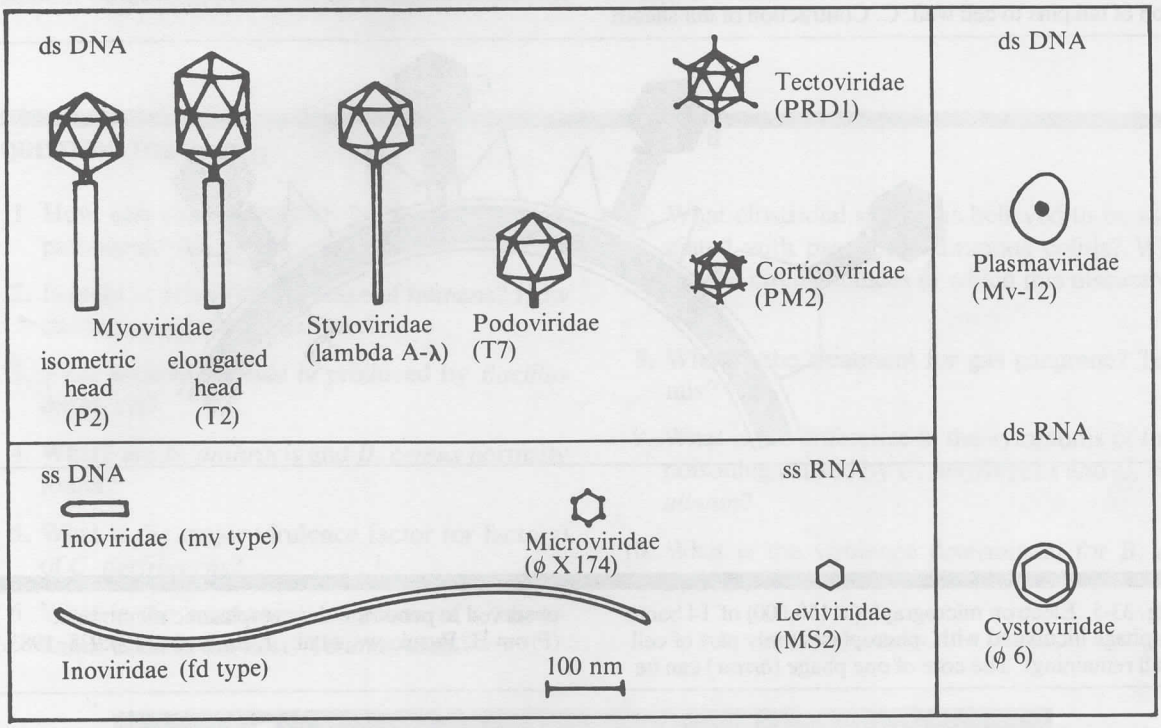
A B C

either single stranded or double stranded. The genome of most bacterial viruses consists of double-stranded DNA. Much of phage research has been directed at the physiology and structure of a species of double-stranded DNA viruses that infects *Escherichia coli*. They are called the *T series* (T1, T2, T3, and so on), and unless otherwise indicated our discussion will be concerned with the T4 virus (Fig. 33-1).

The DNA of the T4 virus is physically and chemically different from that of the host it infects. Physically, the viral DNA is smaller (1.5×10^8 daltons) than the DNA of *E. coli* (3×10^9 daltons). Viral DNA possesses the same bases as other nucleic acids, but there are some variations. Cytosine, for example, is in the form of hydroxymethyl cytosine (HMC) with glucose covalently bonded to it. This is a very important modification that enables the viral DNA to enter the bacterial cell and to evade host nucleases. The viral nucleic acid is surrounded by a protein coat that, in simple viruses, consists of many identical protein molecules, or in complex viruses may consist of different proteins. The protein shell surrounding the nucleic acid is called the *capsid*; the combination of nucleic acid and protein capsid constitutes the *nucleocapsid*. The capsid is made up of individual protein units called *capsomeres*. The arrangement of the capsomeres determines the shape of the virus, which may be *helical* (rod shaped) or *icosahedral* (also called *cubical* or *isometric*). *Icosahedral* refers to a 20-sided object (Fig. 33-2). A complete viral particle may also possess additional components surrounding the protein capsid. This coat that covers the nucleoscapsid is called an *envelope* and is composed of lipid. With very few exceptions bacterial viruses do not possess an envelope; however, many animal viruses do (see Lipids, under Animal Viruses). Attached to the icosahedral head of the T4 bacteriophage are the tail components: collar, core, sheath, baseplate, and tail fibers (see Fig. 33-1). Their role in the infection process will be discussed.

The majority of bacteriophages have a cubical-head-and-helical-tail type of symmetry that is char-

Fig. 33-3. Families of viruses that infect bacteria. (ss = single-stranded; ds = double-stranded.)



acteristic of the T series of bacteriophage. The structure of the various families of viruses infecting bacteria is illustrated in Figure 33-3. Some have short tails, others long tails, and some no tail.

PHYSIOLOGY OF VIRUS INFECTION

Three things can happen when a phage and a bacterial suspension are incubated together: (1) No infection occurs because the virus is not specific for the bacterial species. (2) Infection proceeds with the production of mature virus and the subsequent lysis of the cell. (3) The virus infects the bacterial cell, *but no mature virus is produced because an* equilibrium between virus and host has been reached, a process called *lysogeny*. Our discussion will begin with the physiology of lytic virus infection, followed by a discussion of the process of lysogeny.

Lytic Bacteriophage Infection

ADSORPTION AND INJECTION. A number of important conditions must be met if the bacterial cell is to be infected by the virus. First, the virus must be specific for the bacterial species. Second, the ratio of virus to bacteria must be correct. A ratio of ten viruses to one bacterium ensures that all the bacteria will be infected. If too many viruses are attached to the bacterium, there may be premature lysis without the production of new virus. Third, the bacte*rial growth medium in which the infection process* takes place must contain the necessary metabolites to support the synthesis of viral molecules in the bacterial cell. It should be pointed out that the virus

Fig. 33-4. Bacteriophage infection process. A. Attachment of long tail fibers of virus to cell wall. B. Adsorption of tail pins to cell wall. C. Contraction of tail sheath and injection of core into cell wall. D. DNA injection through core into cytoplasm of bacterial cell.

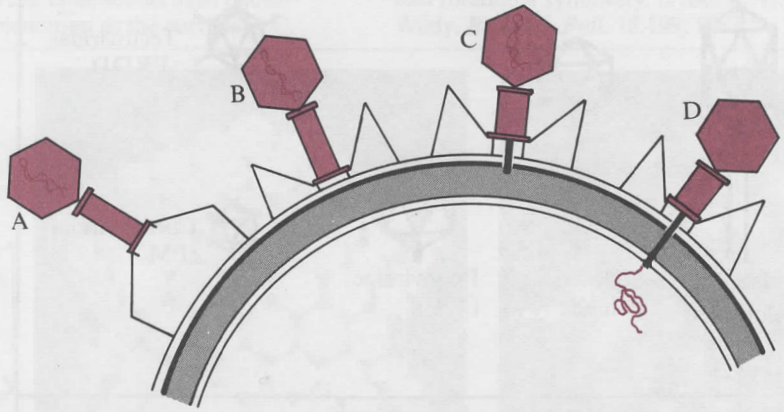
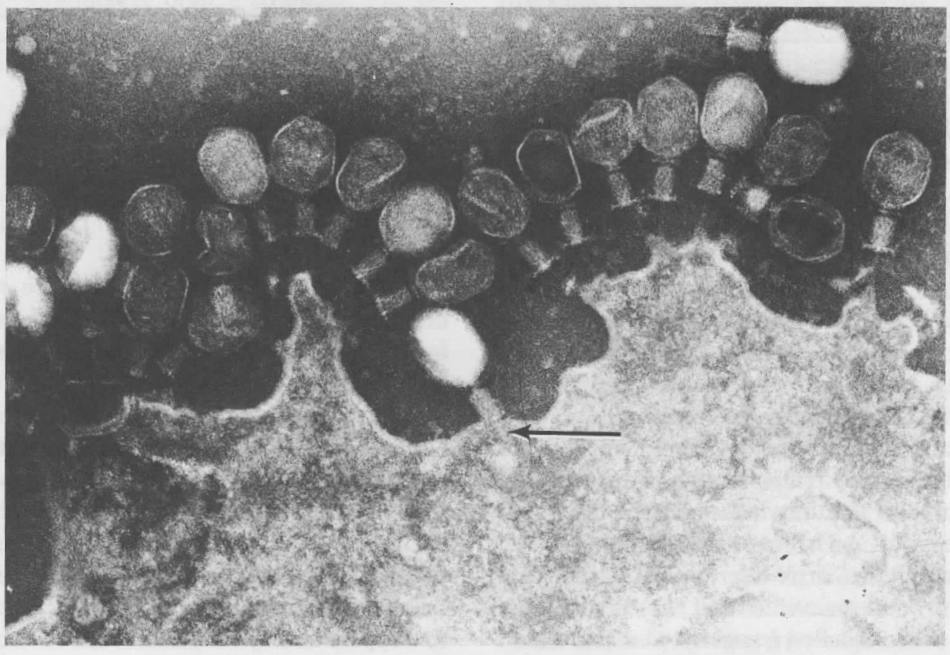


Fig. 33-5. Electron micrograph (× 150,000) of T4 bacteriophage incubated with spheroplasts (only part of cell wall remaining). The core of one phage (*arrow*) can be observed to penetrate the cytoplasmic membrane. (From H. Furukawa, et al., *J. Bacteriol.* 154:938, 1983.)



does not contain or produce any ribosomes or the usual biosynthetic or catabolic enzymes found in the bacterial cell. Because the viral DNA is so small, it carries only enough information for the synthesis of the major structural components plus a few enzymes. The virus must therefore rely on the host's biosynthetic material and machinery to produce more viral particles.

The ends of the phage tail fibers have a chemical affinity for lipoprotein in the cell wall of the bacterium. Attachment of only one tail fiber to the bacterial cell wall is sufficient to initiate binding of virus (Fig. 33-4). Irreversible binding of virus, however, requires the attachment of other tail fibers to the bacterial surface. The binding of tail fibers brings about the contact of the baseplate with the bacterial surface. This is followed by contraction of the tail and passage of the tail tube or core through the cell wall and cytoplasmic membrane (Fig. 33-5). DNA is injected into the cytoplasm of the cell when these two bacterial structures have been punctured.

MATURATION. Once inside the cell, viral DNA is transcribed in a prescribed manner, with the formation of early, middle, and late proteins. Although the maturation process is divided into three periods, some events overlap. One of the early events is the transcription of genes for repairing the bacterial cell membrane that was partially degraded during injection of viral DNA into the cytoplasm. To replicate, the viral DNA must code for genes that are involved in inhibition of host functions and metabolism. One important modification process by T4 involves the alteration of host RNA polymerase so the enzyme recognizes only viral DNA promoters and not host DNA promoters. Phages also code for nucleases that degrade DNA lacking hydroxymethyl-cytosine; thus bacterial DNA is degraded while viral DNA is protected. The nucleotides released from the degraded host DNA are used as precursors for phage DNA. The phage genome also codes for DNA polymerase and other enzymes that are involved in synthesis of deoxyribonucleoside triphosphates. Thus the major early phage functions are concerned with synthesis of viral DNA.

The middle and late periods in viral maturation are involved in transcription of progeny viral DNA (the early proteins were coded on parental DNA). The most important proteins produced during the late period are structural proteins; thus head, tail, and tail fiber synthesis occurs during this period. First, there is assembly of the baseplate, which leads to assembly of tail tube and sheath. The head is assembled separately and into it the phage genome is packaged. Finally the head is attached to the tail and the assembled tail fibers are bound to the phage particle (Fig. 33-6).

RELEASE. Once all of the phage components have been assembled, the mature phage particles must be released from the cell if they are to infect progeny cells. T even phages (T2, T4, and so on) carry a gene that codes for the enzyme lysozyme. Lysozyme attacks the peptidoglycan layer of the bacterial cell wall, causing lysis of the cell and release of virus in a single burst.

Bacteriophage infection can be assayed in the laboratory in the following way. A sample of phage is mixed with a drop of a culture of bacteria plus 1 to 2 ml of soft agar at 44°C. The mixture is shaken slightly and then poured over the surface of hard agar in a Petri dish. The soft agar is allowed to set and is then incubated overnight. During the incubation period bacteriophages diffuse through the agar until they find a bacterium to infect. Each infected bacterium produces progeny phage after 30–45 minutes (depending on the temperature and the type of phage), and lysis occurs with release of phage. Several hundred phage are released from each bacterium, and they in turn infect adjacent bacteria, which repeat the infection cycle, lyse, and release phage. Uninfected bacteria multiply and produce a lawn over the agar surface except at those sites where phage-infected bacteria have lysed. These areas of lysis appear clear and are called *plaques* (Fig. 33-7). The number of plaques coincides with the number of phage in the original sample that infected a bacterium. Plaques are also formed during animal virus infection (see Plaque Assay later in this chapter).

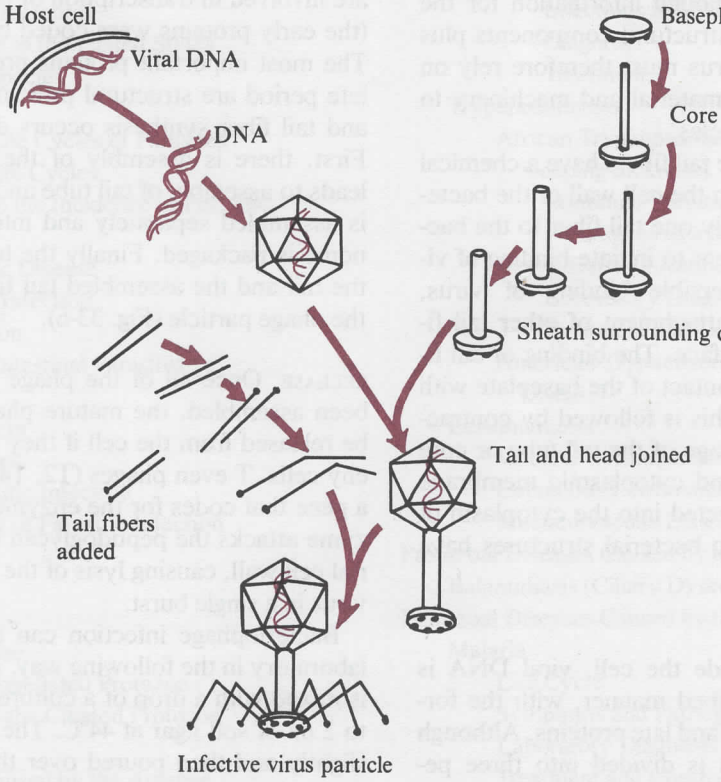


Fig. 33-6. Abbreviated scheme showing assembly pathways of bacteriophage. Each pathway leads to the assembly of a phage component. The components are then assembled into a mature viral particle.

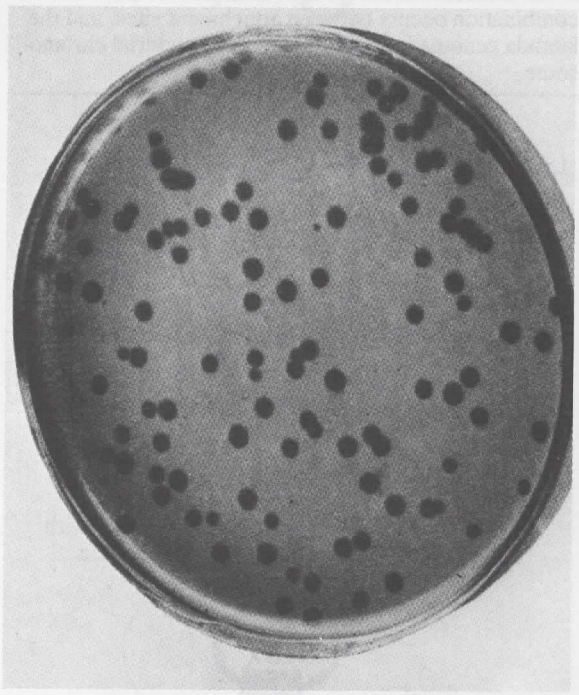
Temperate Bacteriophage Infection (Lysogeny)

Some viruses have evolved mechanisms that control their replication and allow them to maintain a stable relationship with their host. This stable relationship between virus and host cell is called *lysogeny*. The cell harboring the virus is called a *lysogen*, and the virus is called a *temperate virus*. Temperate virus DNA may integrate into the host cell (Fig. 33-8), or it may remain physically independent of the host genome. In either case replication of the phage genome is coordinated with host genome replication; thus at cell division both bacterial cells receive at least one copy of the viral genome. The expression of the replicative mechanisms of the virus is controlled by a repressor that is coded by a phage gene. In its nonproductive state the genes of the temperate virus (except the repressor gene) are not expressed because of the binding of repressor to certain sites on the phage DNA. The presence of the repressor also makes the lysogenic cell immune to infection by a second phage particle of the same phage species.

If the lysogenic cell is treated with ultraviolet light, x-rays, alkylating agents, or carcinogenic chemicals, the phage genome is released from the effects of the repressor and a productive infection ensues in which mature phage particles are assembled and released from the cell (Fig. 33-8).

One of the most widely studied temperate vi-

Fig. 33-7. Bacterial plaques. Agar culture medium shows a lawn of *Escherichia coli* growth on which T2 bacteriophages have produced plaques. (From *Molecular Biology of Bacterial Viruses* by Gunther S. Stent. W. H. Freeman and Company. Copyright © 1963.)



ruses is called *lambda* (λ). During lysogenization lambda becomes inserted into the host genome as a *prophage* (Fig. 33-9). Lambda DNA has been used as a vector for molecular cloning (see Chapter 5). Once inserted, phage DNA and its replication mechanisms are regulated by the systems that control replication of the bacterial chromosome. Some prophages are capable of changing the cell's phenotype, a process called *phage conversion* or *lysogenic conversion*. Some examples in which lysogenic conversion has contributed to the infectious disease process are:

1. *Corynebacterium diphtheriae* is a pathogen whose virulence is related to its ability to produce a toxin. The organism is virulent only when it carries a temperate virus. If the organism is cured of the temperate virus, it is no longer pathogenic. Thus the gene for toxin production apparently resides in the viral genome and not in the host's DNA.
2. Only the streptococci carrying a temperate phage can produce the erythrogenic toxin associated with scarlet fever.
3. *Clostridium botulinum* type C in the lysogenic state is nontoxigenic.
4. Some lysogenic bacteria can be distinguished from nonlysogenic ones. Species of *Salmonella* when infected by a temperate virus will eventually show changes in their surface components (O antigens). The virus in some way prevents the formation of normal cell surface components. For this reason the antigenic components of the lysogenized cell are different from those of the nonlysogenized cell, and this difference can be detected serologically.
5. The lysogenic state of bacteria is rather similar to the relationship between an animal tumor virus and its host. In both instances the viral DNA is incorporated into the host's genome. This is examined further under Animal Viruses.

BACTERIOPHAGES AND TRANSDUCTION

Transduction is a mechanism that, like transformation and conjugation, can alter the phenotype of the cell. Transduction basically involves the ability of a virus to transfer bacterial genes from one bacterial cell to another. Two types of transduction can be distinguished: *generalized* and *restricted.*

Generalized Transduction

Generalized transduction implies that any bacterial gene can be incorporated into the phage head during the infection process. In this technique bacteria are infected with a lytic virus or a lysogenic cell is induced to produce lytic virus. During the maturation process, degraded bacterial DNA may be packaged into the phage head instead of phage DNA (Fig. 33-10). The amount of bacterial DNA incorporated into the phage head is usually equivalent to the amount of phage DNA carried in the

Fig. 33-8. Process of lysogeny and its conversion to the lytic state. A. Virus adsorbs to the cell. B. Viral DNA (in color) is injected into the bacterial cell and becomes circular. C. Viral DNA is incorporated into bacterial DNA (prophage state). The bacterial cell is now lysogenized. D. The lysogenized bacterial cell, having been treated with ultraviolet light, releases viral DNA from the bacterial genome. E. Virus behaves as a lytic virus in the bacterial cell; viral components are produced, and bacterial DNA is degraded. F. Mature viral particles are assembled. G. Viral particles are released upon lysis of the bacterial cell.

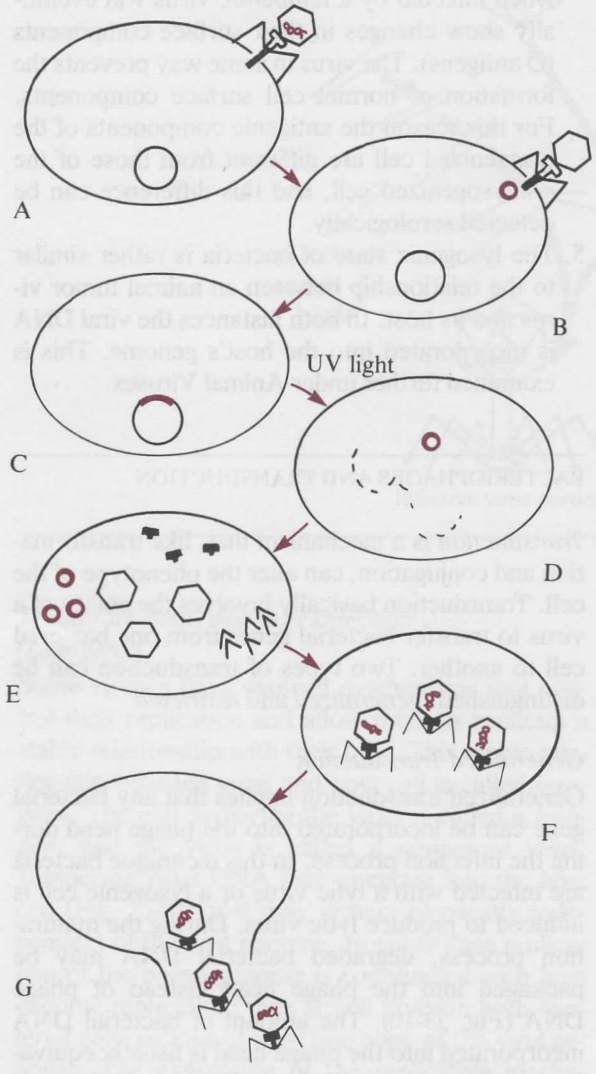
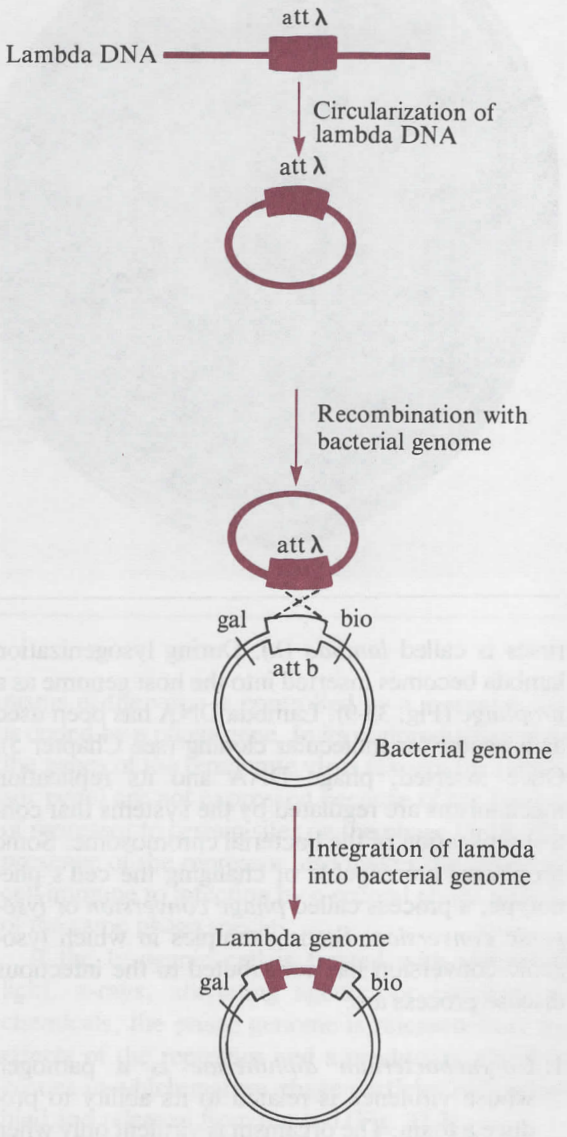


Fig. 33-9. Integration of lambda into host chromosome. In the host, lambda (λ) DNA circularizes. This is followed by interaction of two homologous sites, the attachment sites of lambda (*att* λ) and of the bacterium (*att* b). Galactose (*gal*) and biotin (*bio*) genes are close to the attachment site on the bacterial chromosome. Recombination occurs between attachment sites, and the lambda genome is integrated into the bacterial chromosome.



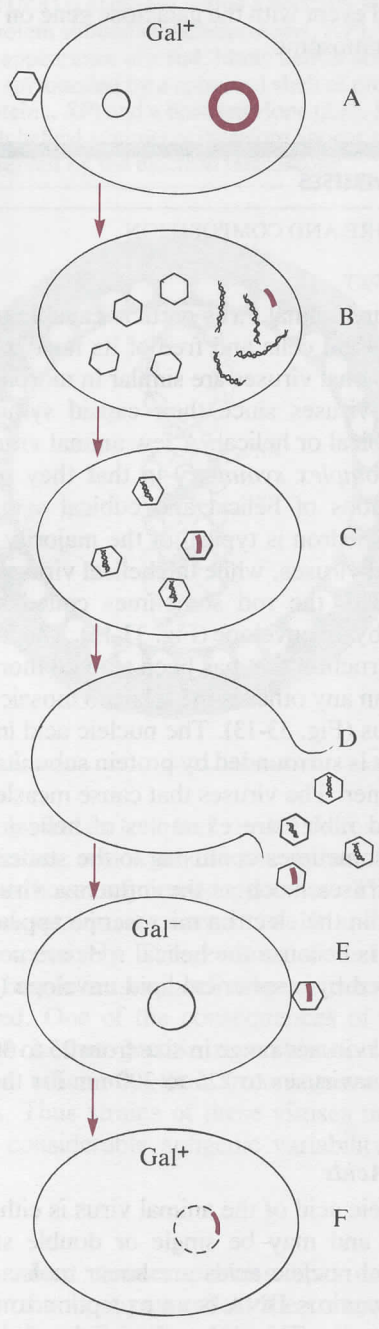


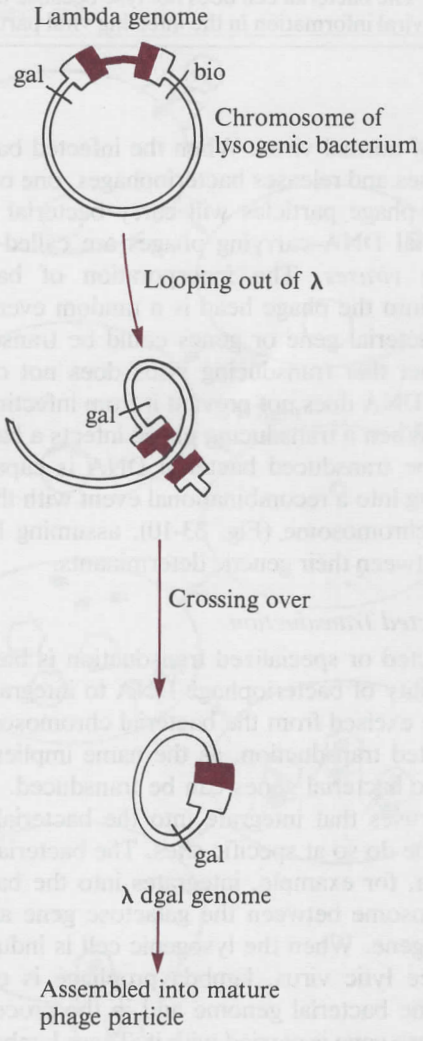
Fig. 33-10. Generalized transduction. A. Bacterial cell with a functional galactose gene (gal$^+$) is infected by a virus. (Bacterial DNA is red; viral DNA is black.) B. Bacterial DNA is degraded C. Bacterial gal$^+$ gene is encased in a viral coat. D. Bacterial cell lyses, releasing virus, one of which carries bacterial gal$^+$ gene. E. Another bacterial cell that carries a nonfunctional galactose gene (gal$^-$) is infected by the transducing virus. F. The transduced gal$^+$ gene engages in recombination with gal$^-$ gene of bacterial chromosome, converting the cell to gal$^+$. The bacterial cell does not lyse because there was no viral information in the infecting viral particle.

head of normal virus. When the infected bacterial cell lyses and releases bacteriophages, one or more of the phage particles will carry bacterial DNA. Bacterial DNA–carrying phages are called *transducing viruses*. The incorporation of bacterial DNA into the phage head is a random event, and any bacterial gene or genes could be transduced. The fact that transducing virus does not contain phage DNA does not prevent it from infecting bacteria. When a transducing phage infects a bacterial cell, the transduced bacterial DNA is capable of entering into a recombinational event with the bacterial chromosome (Fig. 33-10), assuming homology between their genetic determinants.

Restricted Transduction

Restricted or specialized transduction is based on the ability of bacteriophage DNA to integrate into and be excised from the bacterial chromosome. In restricted transduction, as the name implies, only specific bacterial genes can be transduced. Bacterial viruses that integrate into the bacterial chromosome do so at specific sites. The bacterial virus lambda, for example, integrates into the bacterial chromosome between the galactose gene and the biotin gene. When the lysogenic cell is induced to produce lytic virus, lambda prophage is excised from the bacterial genome and in the process the galactose gene is carried with it. These lambda particles are called λ *gal*. Sometimes the excised λ gal lacks certain genes essential for lytic growth. These defective particles are called λ *dgal* (Fig. 33-11). If

Fig. 33-11. Restricted transduction. Integrated lambda DNA is closely associated with the galactose (*gal*) gene in the bacterial chromosome. Deintegration of the lambda genome causes it to loop out and engage in an incorrect recombination with attachment sites (in color) and the gal gene is carried on the viral genome. The defective virus genome (λ dgal) does not carry sufficient viral information for assembly into mature phage particle during the next infection cycle.



we use λ dgal particles to infect other bacteria, the transduced galactose gene can enter into a recombinational event with the galactose gene on the bacterial chromosome.

ANIMAL VIRUSES

STRUCTURE AND COMPOSITION

Morphology

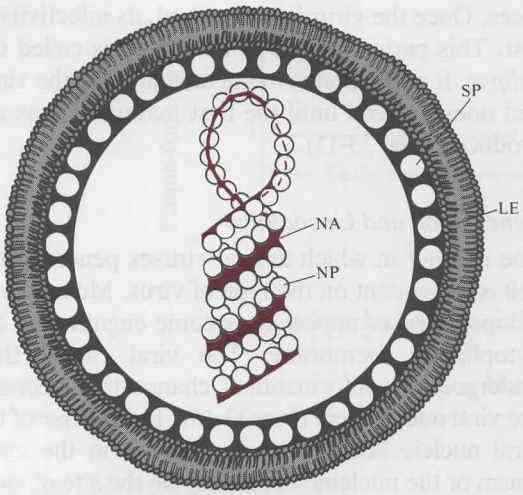
The mature animal virus particle capable of infecting vertebrate cells and free of its host is called a *virion*. Animal viruses are similar in morphology to bacterial viruses since their capsid symmetry is either cubical or helical. A few animal viruses also exhibit *complex symmetry* in that they represent combinations of helical and cubical symmetries. The icosahedron is typical of the majority of cubical animal viruses, while the helical viruses are rod shaped with the rod sometimes coiled and surrounded by an envelope (Fig. 33-12). The helical virus construction that has been studied more extensively than any other is the tobacco mosaic virus, a plant virus (Fig. 33-13). The nucleic acid in the helical virus is surrounded by protein subunits in a helical manner. The viruses that cause measles, influenza, and rabies are examples of helical viruses. What is sometimes confusing to the student is that helical viruses, such as the influenza virus, when observed in the electron microscope appear spherical; this is because the helical rods are coiled and surrounded by a spherical lipid envelope (see Fig. 33-12).

Animal viruses range in size from 25 to 30 nm for the picornaviruses to 225 to 300 nm for the poxviruses.

Nucleic Acids

The nucleic acid of the animal virus is either DNA or RNA and may be single or double stranded. Most viral nucleic acids are linear molecules, but the papovavirus DNA is an exception to this rule and is circular. The molecular weight of animal virus nucleic acids ranges from 1.5 to 3 $\times$ 10^6 daltons

Fig. 33-12. Morphology of a helical virus. The viral nucleic acid (*NA*) is enclosed within a helically shaped core of protein subunits (nucleoproteins, *NP*), giving the virus the appearance of a rod. Many helical animal viruses are surrounded by a spherical shell of protein (shell proteins, *SP*) and a lipid envelope (*LE*). Some viruses with helical symmetry therefore appear spherical when observed by the electron microscope.

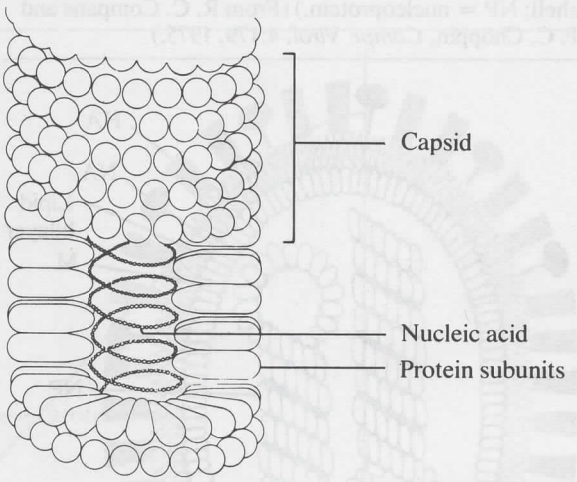


for picornaviruses to 1 to 2 $\times$ 10^8 daltons for the largest viruses, which are the poxviruses. Some viruses also have segmented genomes. The reoviruses and rotaviruses consist of 10 and 11 segments, respectively. The genomes of the influenza viruses, bunyaviruses, and arenaviruses are also segmented. One of the consequences of this segmentation is that genetic recombination can occur more efficiently than in unsegmented viral genomes. Thus strains of these viruses may demonstrate considerable antigenic variability (see p. 737).

Lipids

Several animal viruses possess lipid envelopes, which for the most part reflect the composition of the membranes of the host. Viral envelope composition will therefore vary from one host to another.

Fig. 33-13. Tobacco mosaic virus, a helical plant virus.



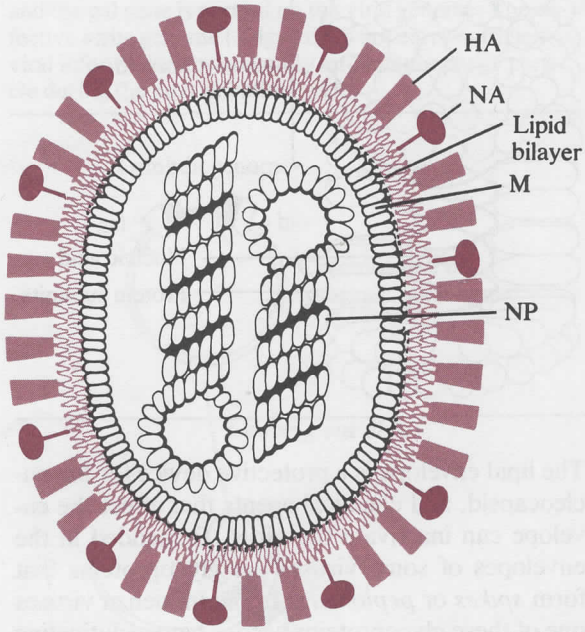
The lipid envelope is a protective device for the nucleocapsid, and chemical agents that affect the envelope can inactivate the virus. Embedded in the envelopes of some viruses are glycoproteins that form *spikes* or *peplomers*. In the influenza viruses one of these glycoproteins carries hemagglutinating activity, while the other carries neuraminidase activity (Fig. 33-14). The latter splits off the terminal sialic acid residue from the oligosaccharide chains on the host's cytoplasmic membrane during the infection process (see Chapter 34 for further discussion of this activity). Both hemagglutinin and neuraminidase activity are located on the same glycoprotein spike in the parainfluenza viruses.

Proteins

The proteins found in the virus are both structural and enzymatic. Structural proteins include those that make up the capsid and the hemagglutinin glycoproteins found in the viral envelope.

Unlike most bacterial viruses, many animal viruses carry enzymatic proteins. Viruses such as the influenza virus and parainfluenza viruses contain the enzyme neuraminidase in the viral envelope. Some viruses, such as the poxviruses and reoviruses, carry RNA polymerase within the capsid.

Fig. 33-14. Influenza virus. (HA = hemagglutinin peplomer; NA = neuraminidase peplomer; M = protein shell; NP = nucleoprotein.) (From R. C. Compans and P. C. Choppin, *Compr. Virol.* 4:179, 1975.)



The poxviruses carry a DNA-dependent RNA polymerase while the reoviruses carry an RNA-dependent RNA polymerase. These enzymes are all virus-specified (coded in the viral genome) and are activated when the capsid of the virus is degraded. The RNA viruses that cause tumors in vertebrates carry a special DNA polymerase called *reverse transcriptase*, which catalyzes the synthesis of a DNA molecule using the RNA genome as a template. (More will be said about this enzyme later in this chapter.)

PHYSIOLOGY OF ANIMAL VIRUS INFECTION

The animal virus infection process can be broken down into four stages: adsorption, penetration and uncoating, maturation, and release.

Adsorption

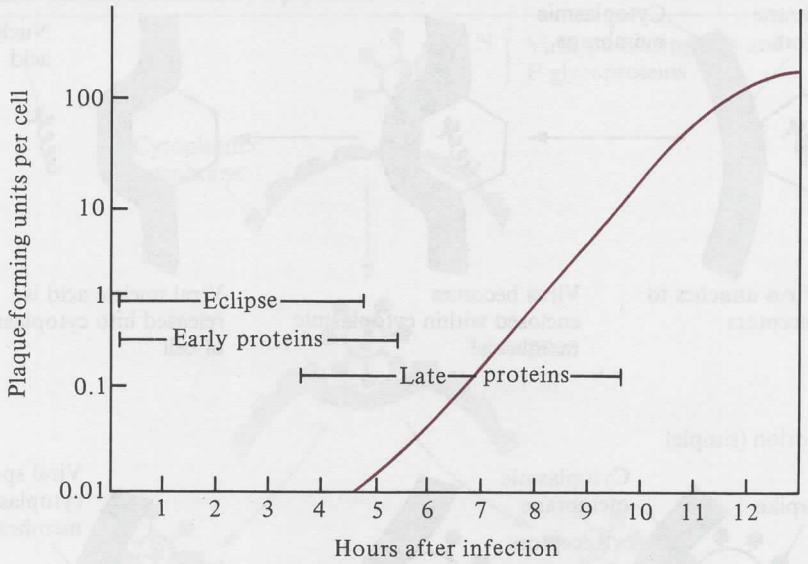
Adsorption of animal viruses to host cytoplasmic membrane appears to involve a chemical interaction between viral proteins and host cell receptor proteins. For nonenveloped viruses the interaction involves a capsid protein; for enveloped viruses it is the viral glycoprotein that acts as a receptor. Alteration of either viral or host surface components reduces the interaction between these two surfaces. Once the virus has adsorbed, its infectivity is lost. This period of loss of infectivity is called the *eclipse*. It corresponds to the uncoating of the virus and does not end until the first mature virions are produced (Fig. 33-15).

Penetration and Uncoating

The manner in which animal viruses penetrate the cell is dependent on the type of virus. Most nonenveloped viruses appear to become engulfed by the cytoplasmic membrane. The viral capsid then undergoes a conformational change that liberates the viral nucleic acid (Fig. 33-16). The release of the viral nucleic acid takes place either in the cytoplasm or the nucleus, depending on the site of virus maturation. It is possible that some nonenveloped viruses fuse with the cytoplasmic membrane and form a phagocytic vacuole that breaks down inside the host cell to liberate the nucleocapsid. Some nonenveloped viruses may be so small that they pass directly through the cytoplasmic membrane without becoming part of a phagocytic vacuole.

Most enveloped viruses gain access to the host cytoplasm by fusion of the envelope with the cytoplasmic membrane. After fusion some viruses appear to liberate the nucleocapsid into the cytoplasm, while other viruses apparently form phagocytic vacuoles (Fig. 33-16). Entry of the nucleocapsid into the cytoplasm is crucial for those viruses (retroviruses for example) that carry their own replicating enzymes within the capsid. A more complex entry is encountered with viruses such as the large poxviruses. The poxvirus is transported into the cell within a phagocytic vacuole, which breaks down and liberates a nucleocapsid core. A special enzyme is then required to degrade the core of the

Fig. 33-15. Growth cycle of animal virus. Mature viral particles do not make their appearance until nearly 5 hours after infection. The maximum number of virus particles appears 12 hours after infection. The time periods will vary from one virus to another.



virus and liberate the viral genome.

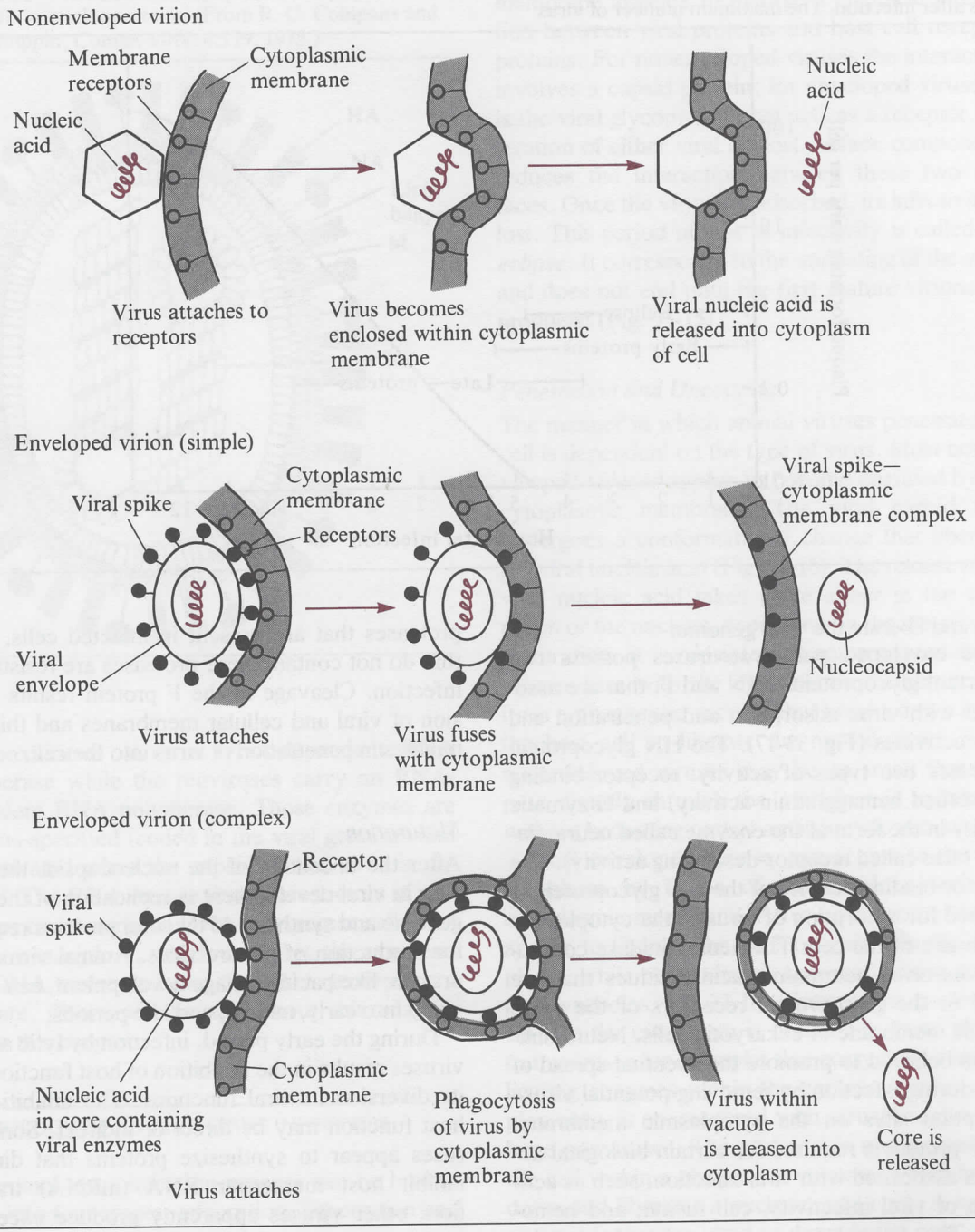
The enveloped paramyxoviruses possess two important glycoproteins, HN and F, that are associated with virus adsorption and penetration and other activities (Fig. 33-17). The HN glycoprotein possesses two types of activity: receptor binding (also called hemagglutinin activity) and enzymatic activity in the form of the enzyme called neuraminidase (also called receptor-destroying activity). The receptor-binding activity of the HN glycoprotein is required for adsorption of virus to the cytoplasmic membrane of the cell. The neuraminidase component dissolves neuraminic acid residues that are found in the glycoprotein receptors of the cytoplasmic membrane of eukaryotic cells. Neuraminidase is believed to promote the eventual spread of virus during infection by removing potential virus–adsorption sites on the cytoplasmic membrane. The F protein is required for certain biological activities associated with viral infection, such as activation of viral infectivity, cell fusion, and hemolysis. The F protein must first be cleaved by proteases that are present in infected cells. Cells that do not contain these proteases are resistant to infection. Cleavage of the F protein results in fusion of viral and cellular membranes and this culminates in penetration of virus into the cell.

Maturation

After the uncoating of the nucleocapsid, the next step in viral development is replication of the viral genome and synthesis of the viral proteins required for production of mature virus. Animal virus maturation, like bacteriophage development, can be divided into early, middle, and late periods.

During the early period, infection by lytic animal viruses results in the inhibition of host function and its diversion to viral functions. The inhibition of host function may be direct or indirect. Some viruses appear to synthesize proteins that directly inhibit host messenger RNA (mRNA) translation; other viruses apparently produce excessive amounts of mRNA that outcompete host mRNA.

Fig. 33-16. Mechanism of penetration and uncoating of animal viruses.



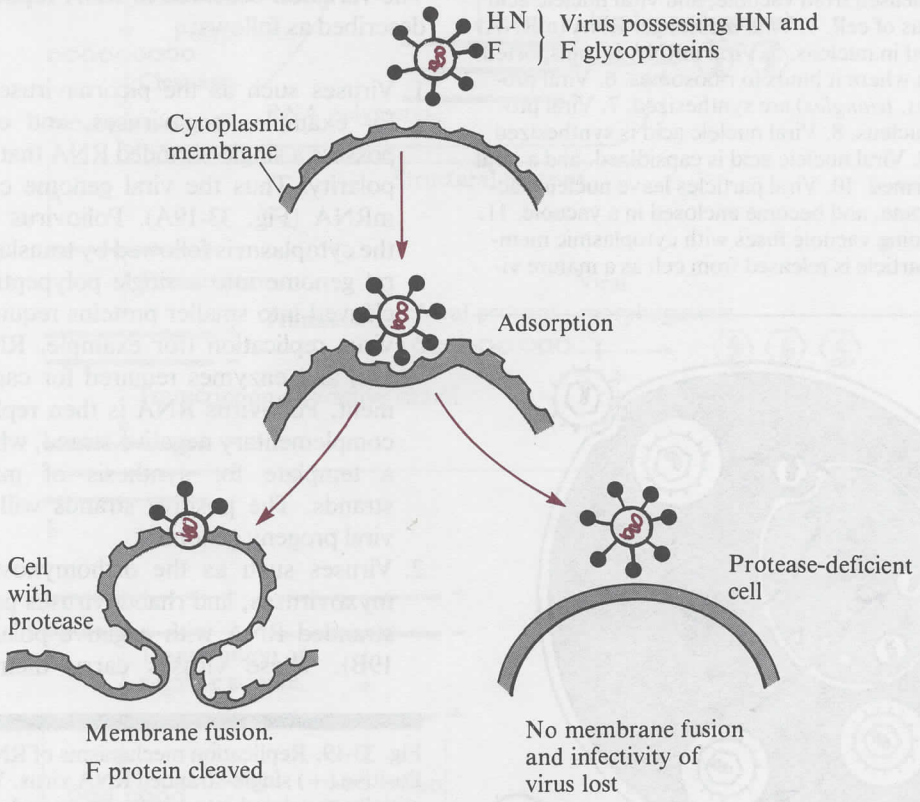


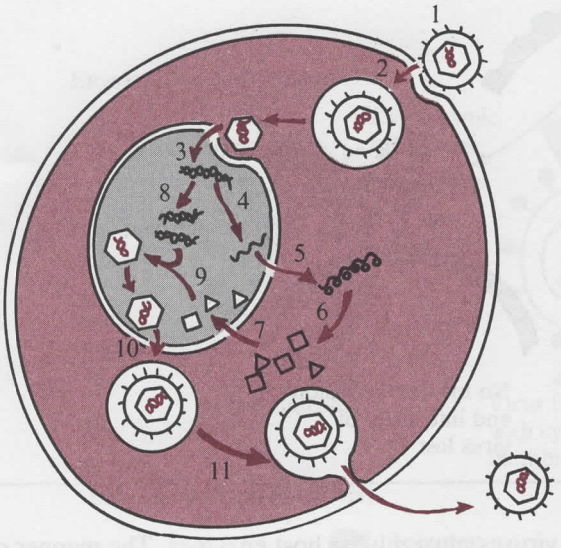
Fig. 33-17. Paramyxovirus penetration of the cell and the role of HN and F glycoproteins. HN glycoprotein is required for membrane attachment. Once attachment has taken place, the F protein is cleaved by a protease, which promotes membrane fusion and infectivity. Cells deficient in protease do not show membrane fusion, and infectivity is lost.

Inhibition of host DNA synthesis is an indirect process brought about by the virus's ability to prevent synthesis of proteins required for DNA synthesis. There is no apparent degradation of animal virus DNA, which is characteristic of bacterial virus development.

The early period is associated not only with inhibition of host function but with the synthesis of viral enzymes for viral genome production. The number of viral enzymes synthesized is greatly dependent on the size of the viral genome. The larger viruses, such as the poxviruses and herpesviruses, rely on the synthesis of their own enzymes, for example, DNA and RNA polymerase, but the smaller viruses must rely on host enzymes. The manner of viral nucleic acid synthesis is dependent on the type of nucleic acid genome as well as the type of virus.

REPLICATION OF DNA VIRUSES. Except for the poxviruses, replication of DNA viruses occurs in the nucleus. Some animal viral DNA's have unusual terminal sequences that make the molecule assume a variety of unusual structures. Consequently transcription of some of the viral DNA genomes may be more complex than the transcription process that occurs in most types of cells. Despite these differences the basic replication process is still similar

Fig. 33-18. Mechanism of infection by a DNA virus that replicates in the nucleus of the cell. 1. Virus adsorbs to cytoplasmic membrane. 2. Virus, during penetration of cytoplasmic membrane, becomes enclosed in vacuole. 3. Virus is released from vacuole, and viral nucleic acid enters nucleus of cell. 4. Viral messenger RNA (mRNA) is synthesized in nucleus. 5. Viral mRNA is transported to cytoplasm where it binds to ribosomes. 6. Viral proteins (*squares, triangles*) are synthesized. 7. Viral proteins enter nucleus. 8. Viral nucleic acid is synthesized in nucleus. 9. Viral nucleic acid is capsidized, and a viral particle is formed. 10. Viral particles leave nucleus, acquire membrane, and become enclosed in a vacuole. 11. Virus-containing vacuole fuses with cytoplasmic membrane, and particle is released from cell as a mature virion.

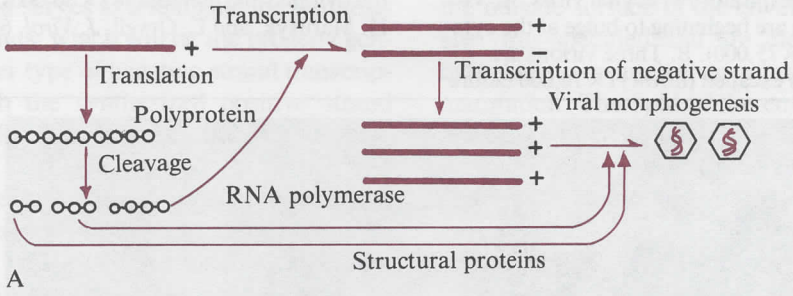


to cellular DNA transcription. An example of the steps involved in the infection process by a DNA virus that replicates in the nucleus is presented in Figure 33-18. As you can observe from this figure the DNA replicated in the nucleus is transcribed into mRNA molecules that are transported into the cytoplasm where the ribosomes are located. Viral proteins synthesized in the cytoplasm enter the nucleus where final virus assembly occurs.

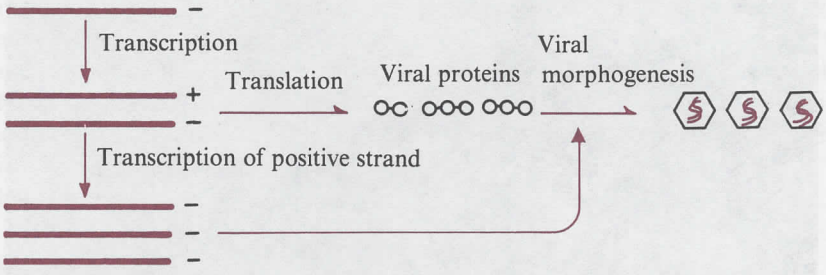
REPLICATION OF RNA VIRUSES. There is a great diversity in the replication of RNA viruses because the viral genome may be single- or double-stranded RNA and because the RNA exhibits polarity, that is, the RNA is either positive or negative stranded. Only positive-stranded RNA can act as mRNA. The variation observed in RNA replication can be described as follows:

1. Viruses such as the picornaviruses (poliovirus for example), togaviruses, and coronaviruses possess a single-stranded RNA that is of positive polarity. Thus the viral genome can act as an mRNA (Fig. 33-19A). Poliovirus uncoating in the cytoplasm is followed by translation of the viral genome into a single polypeptide, which is cleaved into smaller proteins required for poliovirus replication (for example, RNA polymerase) and enzymes required for capsid development. Poliovirus RNA is then replicated into a complementary negative strand, which serves as a template for synthesis of many positive strands. The positive strands will become the viral progeny genomes.
2. Viruses such as the orthomyxoviruses, paramyxoviruses, and rhabdoviruses possess single-stranded RNA with negative polarity (Fig. 33-19B). These viruses carry their own RNA

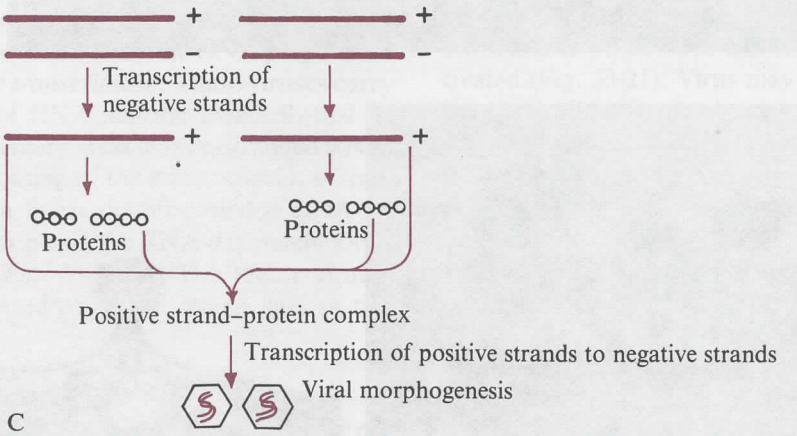
Fig. 33-19. Replication mechanisms of RNA viruses. A. Positive (+) single-stranded RNA virus. Viral genome is initially translated into a polyprotein and cleaved into individual proteins, one of which is RNA polymerase. The RNA polymerase is used to transcribe the viral genome into a negative strand. The negative strand is transcribed into many positive strands, which will become the genomes for viral particles. B. Negative-stranded RNA virus is transcribed into a positive strand. The positive strand is used for translation into viral proteins and is also transcribed into several negative strands, which will become the genomes for viral particles. C. Double-stranded RNA virus. The negative strand is transcribed into positive strands, which are translated into proteins. The mRNA's form complexes with proteins, and then the positive strands are transcribed into negative strands. D. Two identical molecules of single-stranded positive-strand RNA. RNA is transcribed into double-stranded DNA by **reverse transcriptase.** DNA is transcribed into genome RNA as well as mRNA for viral proteins.


Transcription
+
Translation
Polyprotein
Cleavage
RNA polymerase
Transcription of negative strand
Viral morphogenesis
Structural proteins

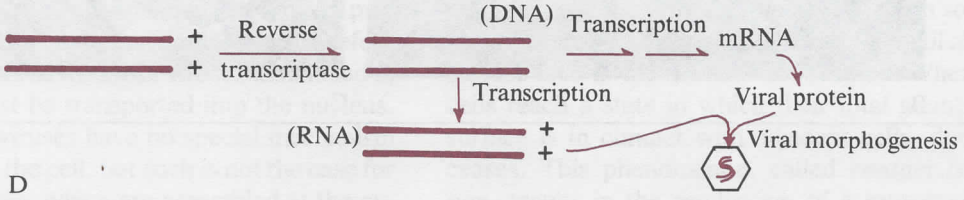
A


Transcription
Translation
Viral proteins
Viral morphogenesis
Transcription of positive strand

B

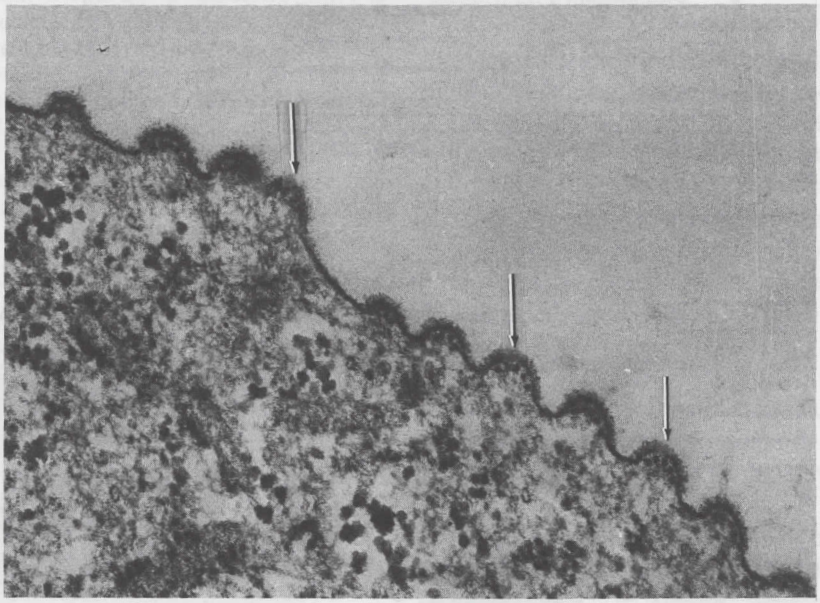

Transcription of negative strands
Proteins
Proteins
Positive strand–protein complex
Transcription of positive strands to negative strands
Viral morphogenesis

C


Reverse transcriptase
(DNA)
Transcription
mRNA
Transcription
Viral protein
(RNA)
Viral morphogenesis

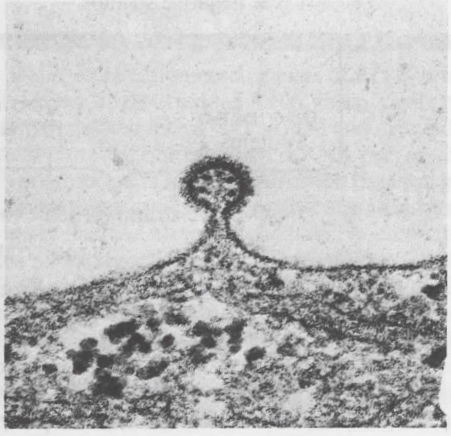
D

Fig. 33-20. Electron micrographs demonstrating the stages of budding of respiratory syncytial virus. A. Virus particles (*arrows*) are beginning to bulge at the cytoplasmic membrane (×75,000). B. Three virions are about to bud; one has escaped (*arrow*) (×76,000 before 8% reduction). C. A virion just before pinching off from the cytoplasmic membrane (×86,000). (From E. Norby, H. Marusyk, and L. Orvell, *J. Virol.* 6:237, 1970.)



A

B



C

polymerase, which transcribes the negative genome into a complementary positive strand. The positive strand serves as a template for synthesis of minus strands, which will be the progeny genomes. Another type of negative strand transcription in which the synthesized positive strand acts as an mRNA coding for viral proteins also takes place.

3. The reoviruses possess a double-stranded RNA in which one strand is positive and the other negative. These viruses are segmented and carry their own RNA polymerase. After the uncoating of the nucleocapsid, the RNA polymerase is activated, which results in transcription of the negative strand of viral RNA into positive-stranded mRNA (Fig. 33-19C). The mRNA's are first translated into proteins but later they form complexes with the viral proteins, and each positive-stranded RNA is transcribed into a negative-stranded RNA. These double-stranded RNA–protein complexes can be transcribed into mRNA molecules, but eventually they will become the mature viral particles.
4. The retroviruses are tumor viruses (also called *oncornaviruses*) that carry the DNA polymerase called *reverse transcriptase*. These viruses carry two strands of RNA that are identical, that is, not complementary. This double-stranded RNA, after the uncoating of the nucleocapsid, is transcribed into a linear double-stranded DNA by reverse transcriptase, the RNA-dependent DNA polymerase (Fig. 33-19D). (The tumor viruses will be discussed at greater length later in this chapter.)

Release

Viruses may be assembled in the cytoplasm or nucleus depending on their site of maturation. All proteins are synthesized in the cytoplasm; therefore the proteins required for those viruses assembled in the nucleus must be transported into the nucleus. Nonenveloped viruses have no special mechanism for release from the cell, but such is not the case for enveloped viruses, which are assembled at the cytoplasmic or nuclear membrane surface. When enveloped viruses pass through the cytoplasmic or nuclear membrane they obtain lipid plus glycoprotein. The glycoproteins appear as projections on the outside surface of the envelope. Glycoproteins are virus-specified and are incorporated into the cytoplasmic or nuclear membrane just before viral maturation. The lipid of the envelope is of cellular origin. The exit of nucleocapsid through the cytoplasmic membrane is called *budding* (Fig. 33-20) and results in the virus's being surrounded by an envelope.

The multiplication cycle for animal viruses varies considerably but is always longer than bacteriophage development. The poliovirus has the shortest multiplication cycle, which varies from 6–8 hours. One of the longest multiplication cycles is that of the adenovirus, in which 48 hours is required before virus is released from the infected cell.

CULTIVATION OF VIRUSES

Viruses can grow only in living cells. An economical and readily available means of cultivation is the embryonated hen's egg. The egg contains many cavities into which virus can be inoculated and cultivated (Fig. 33-21). Virus may be inoculated onto the chorioallantoic membrane, where some viruses are capable of producing local lesions (pocks). Inoculation of the embryo has been used for the production of virus for vaccines, most notably the influenza vaccine.

Because the cells of numerous organs can now be grown in vitro, most viruses are today cultivated in what is called *cell culture*. Thus animal cells can be grown just as bacterial cells can, even though an animal cell's chemical and environmental requirements are much more stringent. When cultured in glass or plastic containers, the cells attach to any available surface and continue to divide until all the surface area in the medium is occupied. When the cells reach a state in which their total membrane surface is in contact with adjacent cells, division ceases. This phenomenon, called *contact inhibition*, results in the production of a monolayer of cells in the culture medium. A number of animal cell lines can then be maintained indefinitely if the

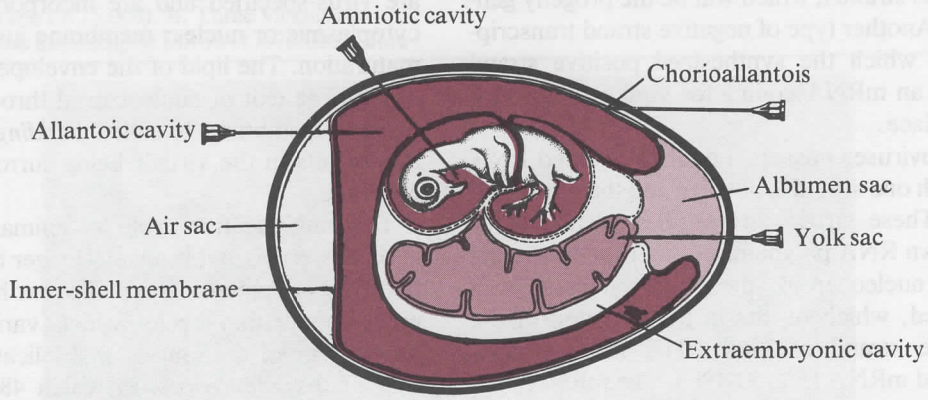


Fig. 33-21. Possible sites of viral inoculation into embryonated egg. (Courtesy Audiovisual Service, Department of Pathology, University of Illinois Medical Center, Chicago.)

originally cultured cells are frozen under special conditions.

There are two types of cell culture: primary and continuous. Primary cultures are cells derived from tissue, such as human and kidney, that is normal and has a finite life span (cannot replicate continuously). Continuous cultures are derived from malignant tissue, which can undergo an unlimited number of replications. The HeLa cell, originally derived from a woman (Henrietta Lacks) who had cervical cancer, is a frequently used cell line. Malignant cells have lost the property of contact inhibition and thus have a tendency to pile up to form tumors (see Viruses and Cancer).

Some viruses will not replicate in cell culture and can be cultivated only in whole animals such as mice, rabbits, and guinea pigs. Experiments involving the immune response, for example, are best carried out in virally infected whole animals. Ordinarily, however, animal inoculation serves merely as a diagnostic tool in the identification of viruses from clinical specimens. In this procedure the animal is inoculated with the clinical specimen and is then observed periodically for signs of illness or is killed so that histological sections of infected tissue can be examined.

EPIDEMIOLOGY

Like other agents, viruses may be spread to humans directly or indirectly. Human-to-human spread may be horizontal or vertical (see Chapter 13). The routes by which viruses enter the human body include the following:

1. Direct contact. Direct contact generally means close physical contact with someone who is a symptomatic or an asymptomatic carrier of the virus. Often it involves contact with oral or genital tract secretions. Kissing, for example, can be a method of transmission of herpes simplex virus type 1, while sexual intercourse may be responsible for transmission of herpes simplex virus type 2. Several viruses can penetrate the placental barrier during pregnancy to cause disease. Viral agents that cause congenital infection were described in Chapter 13, Table 13-14.
2. Respiratory route. Infections of the respiratory tract accompanied by coughing and sneezing are sources for transmission of virus to others. The droplets produced contain virus particles that may be inhaled directly into the upper respiratory tract. Viruses associated with the re-

Table 33-1. Viral Diseases Affecting the Respiratory Tract or Acquired Through the Respiratory Tract

Virus	Primary site of infection and/or disease is respiratory tissue	Type of respiratory disease	Type of nonrespiratory disease
Influenza virus	Yes	Influenza, pneumonia	—
Parainfluenza viruses	Yes	Croup, bronchitis, common cold	—
Adenoviruses	Yes	Pharyngitis, rhinitis	—
Respiratory syncytial virus	Yes	Infant bronchiolitis, pneumonia	—
Rhinoviruses	Yes	Common cold, croup, bronchitis	—
Coxsackieviruses	Yes	Bronchopneumonia, common cold	Aseptic meningitis
Echoviruses	Yes	Common cold	Aseptic meningitis
Measles virus	No	—	Systemic disease
Mumps virus	No	—	Systemic disease
Rubella virus	No	—	Systemic disease
Varicella-zoster virus	No	—	Systemic disease
Epstein-Barr virus	No	—	Systemic disease
Smallpox (variola) virus	No	—	Systemic disease
Lassa fever virus	No	—	Systemic disease
Marburg virus	No	—	Systemic disease

Table 33-2. Viral Diseases Affecting the Gastrointestinal Tract or Acquired Through the Gastrointestinal Tract

Virus	Primary site of disease is gastrointestinal tract	Type of nongastrointestinal disease	Type of gastrointestinal disease
Rotaviruses	Yes	—	Epidemic gastroenteritis and infantile diarrhea
Coxsackieviruses	No	Aseptic meningitis	—
Echoviruses	No	Aseptic meningitis	—
Poliovirus	No	Poliomyelitis	—
Hepatitis A virus	No	Hepatitis	—

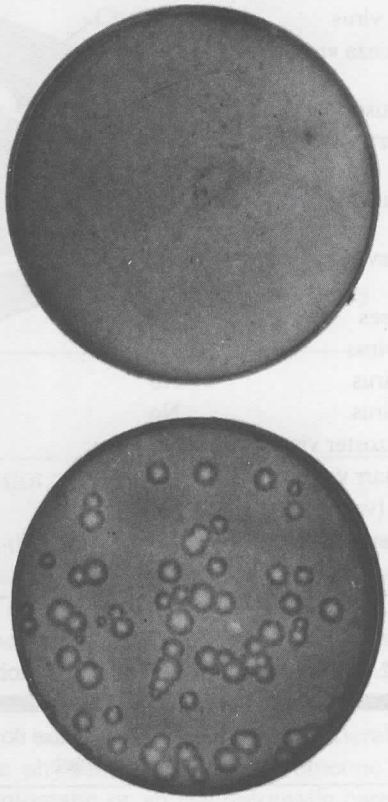
spiratory tract are outlined in Table 33-1.

3. Alimentary route. Several viruses that infect the intestinal tract, for example, the enteroviruses and hepatitis A virus, are excreted in the feces (Table 33-2). Excreted virus may contaminate water supplies and serve as a means of transmission by the fecal-oral route.
4. Skin. Some viruses are transferred by injection through the skin (Table 33-3). For example, hypodermic injection or blood transfusion is a common avenue for transmission of hepatitis B virus and some herpesviruses (such as the cytomegalovirus and the agent that causes mononucleosis). A few viruses are transmitted by arthropod bites, but most of these infections occur in lower animals.

Table 33-3. Viral Diseases Acquired Through the Skin or Causing Lesions of the Skin

Virus	Acquired through the skin	Disease manifestation
Herpes simplex virus type 1	Yes	Cold sores
Papillomaviruses	Yes	Wart
Molluscum contagiosum virus	Yes	Molluscum contagiosum
Smallpox virus	No	Rash
Measles virus	No	Rash
Varicella-zoster virus	No	Rash (chickenpox, shingles)
Coxsackieviruses	No	Rash
Echoviruses	No	Rash
Hepatitis B virus	Yes (by injection)	Hepatitis

Fig. 33-22. Animal viral plaques. Plaques of a strain of influenza virus before and after 5 days of incubation on human amnion cells. (From A. Sugiura and M. Ueda, *J. Virol.* 7:499, 1971.)

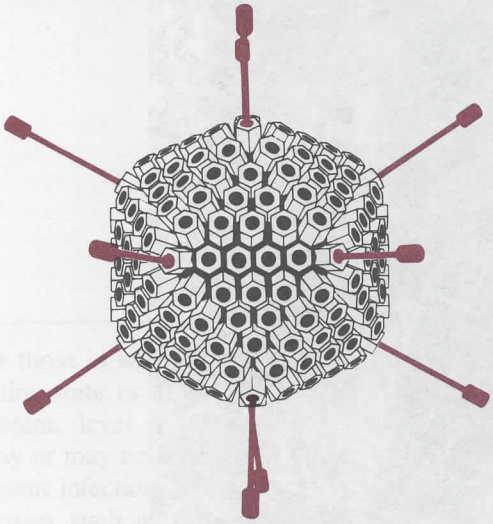


EFFECTS OF VIRAL INFECTION ON THE HOST CELL

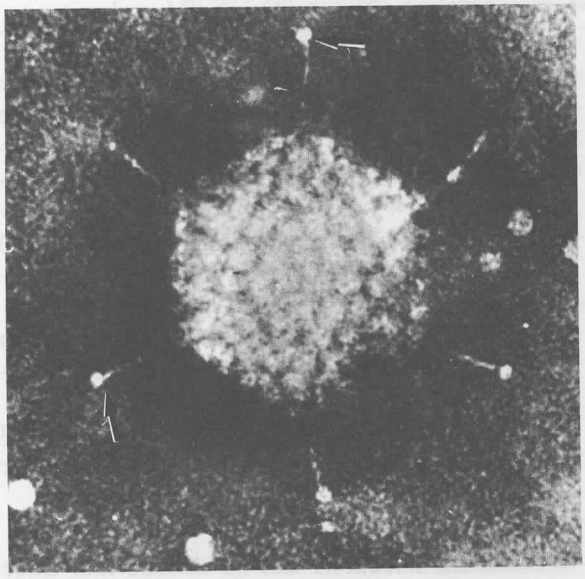
Cytopathic Effects

Some of the biochemical changes caused by viral infection, such as those affecting cellular macromolecular synthesis, were discussed previously. The most frequent effect of virus infection is lysis of the cell. Lytic viruses produce *cytopathic effects* that may be observed macroscopically or microscopically. The most recognizable cytopathic effect is the formation of plaques, which are cleared areas on a monolayer of cells resulting from the death of cells, and which resemble bacterial plaques (Fig. 33-22). The lysis of cells in the infected animal can result in temporary or permanent loss of function. The crippling effects of poliovirus, for example, are the result of lysis of cells of the nervous system. The actual cause of cytolysis after viral infection is not known, but several changes occur in the cell that together may be responsible for this effect. There are nuclear changes, which may include chromosomal breakage, the appearance of fragments in the cell nucleus, and shrinkage of the nucleus. Cytoplasmic membrane changes also occur in virus-infected cells. One of these causes cells to pile up owing to loss of the property of contact inhibition. The trigger for lysis of the cell may be the release of lysosomal enzymes such as phospholipase that break down the cytoplasmic membrane.

Viruses do not produce toxins; therefore any cytotoxicity must reside in the structural components of the virus. Only one or two examples of cytotoxicity have been described. One of these involves the penton fibers that are attached to the capsid of the adenovirus (Fig. 33-23).



A



B

Fig. 33-23. Adenovirus. A. Model of adenovirus showing capsomere arrangement and placement of the 12 pentons (only 10 are shown). Each penton consists of a penton capsomere, a fiber, and a terminal knob. (From R. W. Horne, I. P. Ronchetti, and J. M. Hobart, *J. Ultrastruct. Res.* 51:233, 1975.) B. Electron micrograph of adenovirus. Note the penton fibers and terminal knobs (*arrows*). (With permission from R. C. Valentine and H. G. Pereira, *J. Mol. Biol.* 13:13, 1965. Copyright by Academic Press Inc. [London] Ltd.)

Inclusion Body Formation

During the replication of certain viruses in the cytoplasm or nucleus of infected cells, inclusion bodies can be demonstrated by histological stains. These inclusion bodies may be viral proteins, viral nucleic acids, mature virions, or simply unusual cellular products resulting from the infection process. Some inclusion bodies are so distinctive that their presence is diagnostic (*pathognomonic*). Viral inclusion bodies associated with infection are those of adenovirus (Fig. 33-24), rabies virus (*Negri bodies*), reovirus, and poxvirus (*Guarnieri bodies*), among others.

Cell Fusion

Many enveloped viruses such as the paramyxoviruses and herpesviruses cause cells to fuse during the infection process. One of the glycoproteins found on the envelope of the paramyxoviruses is called the F protein, as was discussed previously. The F protein, which is responsible for membrane fusion, appears also to be responsible for fusion of adjacent cells at the site of infection. Cell fusion results in the formation of giant cells or syncytia in which a single membrane surrounds a mass of cytoplasm containing many nuclei (Fig. 33-25).

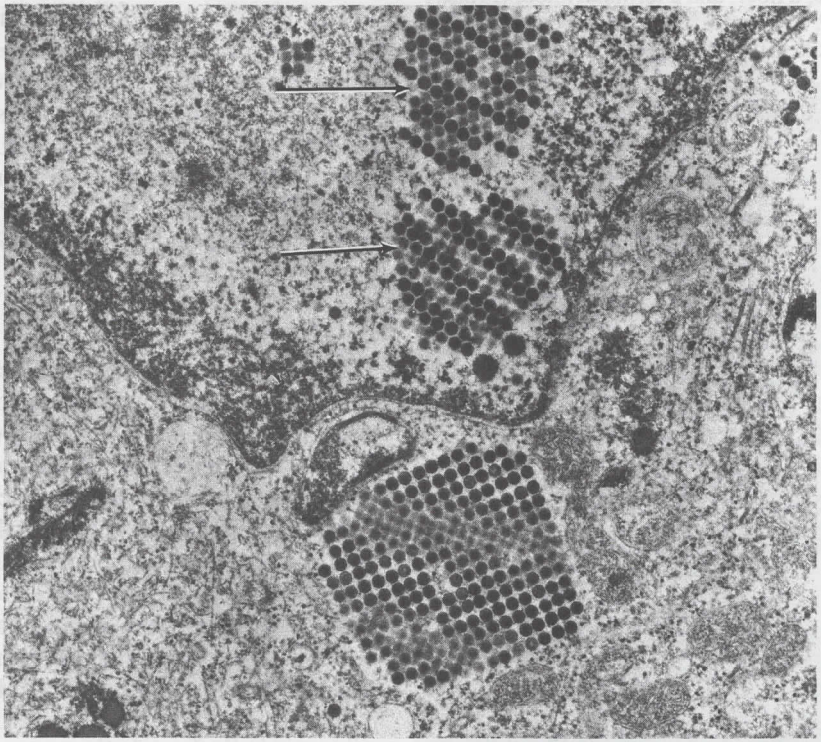
Changes in Surface Antigens

During viral infection there are changes in the antigenic character of the cell surface. One change that is always found during infection by enveloped viruses is due to incorporation of virus-specified glycoproteins into the cytoplasmic membrane. Nonenveloped viruses also code for proteins that are associated with the cell surface. These virus-specified proteins induce both humoral and cell-mediated immunity in the host. Some of these virus-specified proteins will be discussed later.

Interferon Production

In cell cultures and in whole animals, soon after viral infection some cells produce a protein that has the ability to prevent the infection of healthy cells by the same virus. This protein, called *interferon*, may be an important host defense mechanism. It is discussed later in this chapter.

Fig. 33-24. Electron micrograph (×30,000 before 30% reduction) of adenovirus inclusion bodies. Several inclusions are seen in the nucleus (*arrows*), and one is free in the cytoplasm. (From R. Wigand, H. Gelderblom, and H. Brandis, *Arch. Virol.* 64:225, 1980.)



Persistent Viral Infections

Most cells infected by viruses will lyse. An animal virus that lyses one type of cell, however, may infect another cell and enter into a state of persistence. Persistent infection may result in the lysis of only a few cells in the culture or organ and the production of small numbers of viruses. One of the consequences of this type of infection is that only a small proportion of cells will ever become infected. Factors that may be associated with persistence are the following:

1. The virus infecting the cell, or its antigens, are not sufficient to stimulate antibody response; for example, the virus that causes kuru is nonimmunogenic (see Slow Virus Diseases in Chapter 34 for further discussion of this disease). In some instances the viral antigens are present on the surface of the cell but do not elicit an effective antibody response. This ineffective antibody response can be referred to as *immunological tolerance*.
2. The infected tissue (for example, nervous tissue) is relatively inaccessible to circulating T lymphocytes or other immunological components.
3. Virus infects lymphocytes, causing an alteration of the immune response by inducing the production of nonneutralizing antibodies.
4. The immune system of the host has been altered by infection, age, underlying illness, or other factors.

Persistent infections can be divided into three types: latent, chronic, and slow. *Latent* infections

Fig. 33-25. Multinucleated giant cell with intracytoplasmic inclusion (*arrow*) produced by respiratory syncytial virus. (Courtesy H. M. Yamashiroya, Department of Pathology, University of Illinois Medical Center, Chicago.)

are those in which the virus persists in a nonreplicating state or at an undetectable, possibly intermittent, level of replication. Clinical symptoms may or may not be evident during latent infection. Latent infections are characteristic of the herpesviruses such as the cytomegaloviruses and herpes simplex. Herpes simplex virus, for example, causes a latent infection that is expressed in the form of intermittent cold sores. *Chronic* infections are those in which the virus causes cytolysis but symptoms are subclinical and do not impair organ function. Hepatitis (hepatitis B virus) and rubella (rubella virus) are examples of chronic infections. *Slow* persistent infections are those in which there is a long incubation period (years) and virus continues to multiply, albeit slowly, causing a protracted, continued destruction of tissue often resulting in death. An example of a slow virus disease in humans is kuru (see Slow Virus Diseases in Chapter 34). Major groups of viruses that cause persistent infections are presented in Table 33-4.

Transformation

Viral transformation (not to be confused with genetic transformation associated with bacteria) is a process associated with tumor viruses. The virus genome integrates into the host's genome and is capable of altering certain host properties and producing tumors (see Oncogenic Viruses and Cancer).

MEASUREMENT OF ANIMAL VIRUSES

Animal viruses, like their bacterial counterparts, can be assayed in the laboratory. The quantitation of virus may be accomplished by measuring the total number of viral particles in a suspension or by measuring the number of particles that can cause an infection (infectious virus).

Infectious Virus

PLAQUE ASSAY. Plaque assay for infectious viruses is similar to the plaque assay for bacterial viruses. Monolayers of susceptible tissue culture cells are inoculated with aliquots from a series of viral dilutions. When a cell has been infected, viral progeny will be produced and released to infect adjacent cells. After several days a localized area of infection, called a **plaque**, appears. One widely used

Table 33-4. Viruses Causing Persistent Infections in Humans

Viral agent	Disease	Effect on host cell
Rubella virus	Fetal anomalies and panencephalitis	Inhibition of cell division
Measles virus	Subacute sclerosing panencephalitis	Cytolysis
Human papillomavirus	Warts	Transformation
Hepatitis B virus	Hepatitis	Immunopathology
Herpes simplex virus types 1 and 2	Herpes	Cytolysis
Varicella-zoster virus	Zoster (shingles)	Cytolysis
Cytomegalovirus	Mononucleosis	Cytolysis
Epstein-Barr virus	Mononucleosis, Burkitt's lymphoma	Transformation
Kuru virus	Kuru (encephalopathy)	Cytolysis
Creutzfeldt-Jakob virus*	Creutzfeldt-Jakob disease (encephalopathy)	Cytolysis
JC virus	Leukoencephalopathy	Transformation
BK virus	?	None
Adenovirus	?	None

*Creutzfeldt-Jakob disease has recently been demonstrated to be caused by an infectious agent (prion) that does not possess a nucleic acid (see Slow Virus Diseases, Chapter 34.)
Source: Adapted from N. Nathanson, D. Schlessinger (Ed.), *Microbiology—1977*. Washington, D.C.: American Society for Microbiology, 1977.

Fig. 33-26. Numerous pocks (*arrows*) produced on chorioallantoic membrane of embryonated hen's egg after 5 days' incubation with virus at 35.5°C. (Courtesy Audiovisual Service, Department of Pathology, University of Illinois Medical Center, Chicago.)

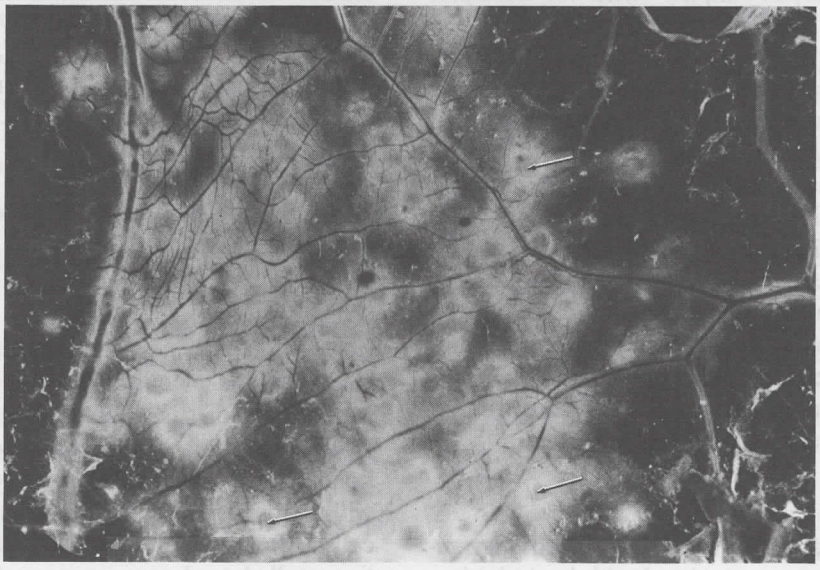
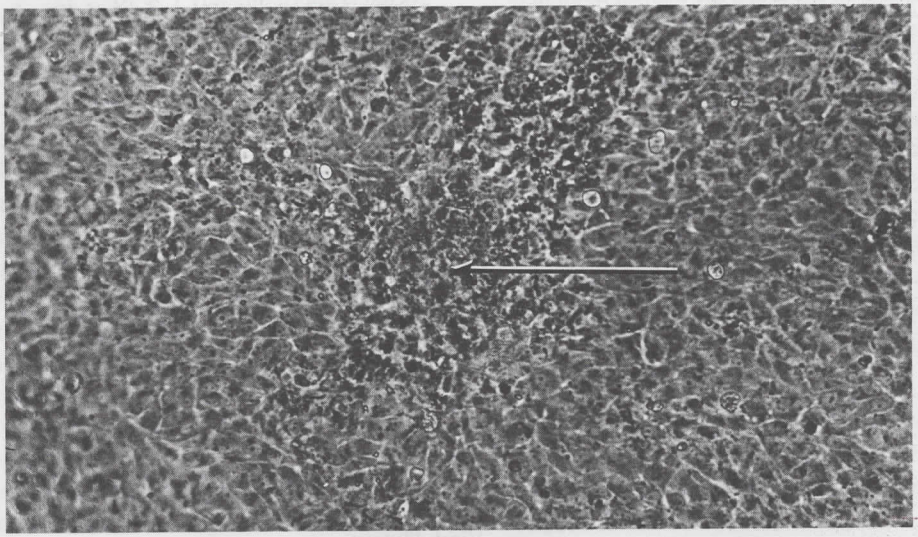


Fig. 33-27. Transformed cell focus (*arrow*) produced by virus. (From K. Hamada, et al., *J. Virol.* 38:327, 1981.)



method for identification of the plaque is based on the differential staining abilities of infected vs. uninfected cells. Neutral red can be used as the differential stain. Some plaques represent areas where the cells have been completely destroyed; these can be viewed as cleared areas (see Fig. 33-22).

POCK FORMATION. When some viruses are cultivated on the chorioallantoic membrane of chick eggs, lesions called *pocks* are produced. The membrane can be removed and spread out and the pocks counted. Each pock represents the original site of a single virus infection (Fig. 33-26).

FOCUS FORMATION. Some viruses that infect cells do not cause visible plaques or pocks. Instead, virus infection stimulates cell proliferation and after a period of time results in the formation of a mass of cells called a *focus* (Fig. 33-27).

SERIAL DILUTION END POINT. The serial dilution end point is an assay that measures the effect of infection by a dose of virus rather than by a single virus. A series of dilutions of the viral suspension is prepared. A certain volume of each dilution is then inoculated into a susceptible animal or cultured cells. A suitable length of time is allotted for the viruses to multiply and produce a measurable effect, after which the infected animals or culture cells are scored. A positive test is indicated when the expected physiological or cytopathic response has occurred. The percentage of positive responses at each dilution is plotted (Fig. 33-28). The titer or end point is expressed as the smallest amount of virus capable of producing an effect on 50 percent of the infected subjects. This is referred to as the ID_{50} (infectious dose producing a 50 percent response) or the LD_{50} (a lethal dose for 50 percent of the animals).

Total Virus

The total number of virus particles in a suspension can be determined by direct or indirect means.

DIRECT MEASUREMENT. Viruses can be counted by electron microscopy. The viral particles are mixed with a known concentration of latex spheres of similar size. The mixture is coated on an electron microscope grid and a count is taken. Since the concentration of spheres is known, the number of virus particles can also be determined (Fig. 33-29).

Fig. 33-28. Determination of the 50 percent end point of a viral suspension. In the graph the highest dilution in which 50 percent of the subjects are shown to be infected is approximately 1×10^{-6}.

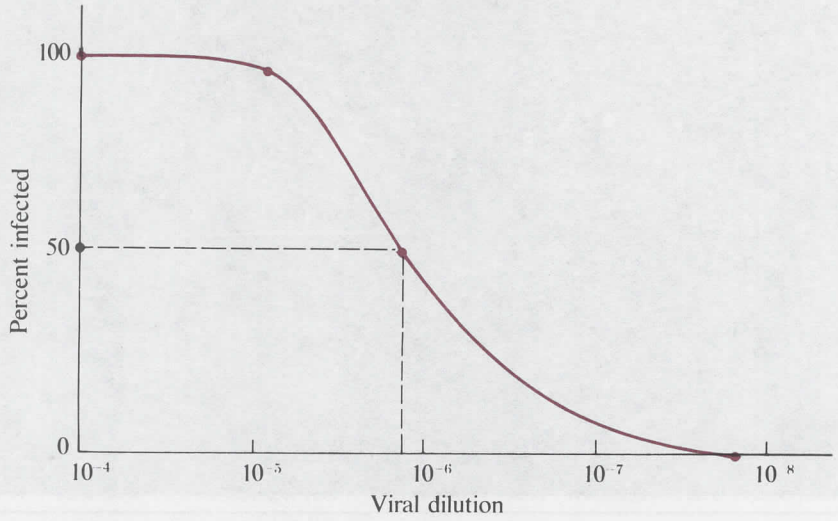
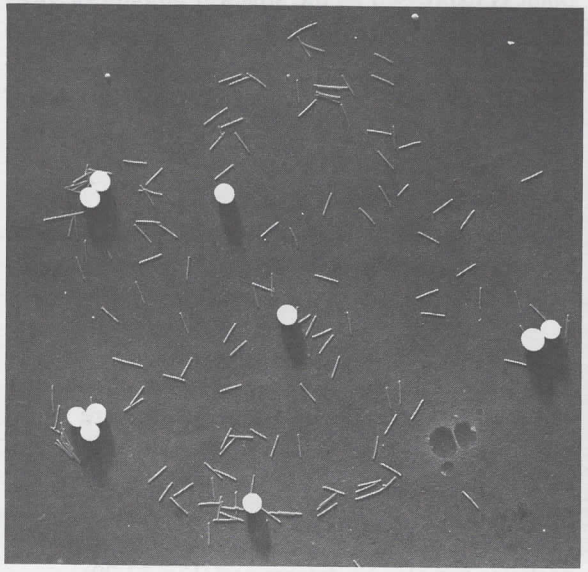


Fig. 33-29. Direct electron microscopical measurement of viral particles mixed with latex spheres. (From J. Symington, B. Commoner, and M. Yamada, *Proc. Natl. Acad. Sci. U.S.A.* 48:1675, 1962.)



INDIRECT MEASUREMENT. Many viruses contain surface factors, for example, capsid proteins, that enable them to bind to erythrocytes. This characteristic forms the basis of the *hemagglutination test*. In the hemagglutination test virus is added to a suspension of red blood cells, and a lattice of red blood cells held together by virus is formed. The maximum number of cells with which a virus can combine is two. The technician looks for the dilution of virus that will just hemagglutinate a specific concentration of red blood cells.

DIAGNOSTIC PROCEDURES IN VIRAL IDENTIFICATION

One problem associated with treatment of viral diseases is the inability to use techniques that provide a rapid diagnosis. Many new techniques are available, but some of them require sophisticated equipment that is not always available to the laboratory. In addition the cost is likely to be prohibitive. Laboratory diagnosis is often unnecessary for viral infections like the usual childhood diseases (for ex-

ample, mumps, measles). Diagnosis does become important when (1) it is suspected that the virus is one associated with a high mortality and its spread in the community could be devastating (for example, smallpox, polio), (2) there is cross infection in the hospitalized patient resulting in severe illness (for example, influenza A, syncytial virus infections), and (3) pregnant women are suspected of carrying viruses that are known to cause abnormalities in the fetus (for example, rubella). At one time even the identification of a virus was of little help because antiviral drugs were unavailable for treatment. More antiviral drugs are now becoming available, and rapid diagnosis of viral agents is becoming more practicable.

Laboratory diagnosis of viral agents, as with other microorganisms, depends on the identification of viral antigens and host antibodies. For relatively simple, reliable, and rapid methods of detecting viral antigens, immunofluorescence and immunoperoxidase tests are best. For the reliable detection of viral antibodies as well as antigens, the enzyme-linked immunosorbent assay (ELISA) is available.

Immunoperoxidase Test

The immunoperoxidase test is similar to immunofluorescence and can be used either directly or indirectly. Instead of a fluorescein-labeled conjugate, an enzyme, usually horseradish peroxidase, is conjugated with the specific antiserum (direct method) or with the nonspecific anti–gamma globulin fraction (indirect method). The test is not useful for the detection of virus-infected cells in respiratory secretions because of the presence of endogenous peroxidase. Other enzymes such as phosphatase are currently being explored as substitutes for peroxidase. The viruses most reliably detected by this method are cytomegalovirus, herpesvirus, and rabies virus. A discussion of this technique can be found in Chapter 16.

Radioimmunoassay

Radioimmunoassay is discussed in detail in Chapter 16. It has been used primarily for the detection of the hepatitis viruses, rotaviruses, and Norwalk agent. The ELISA is now replacing this method.

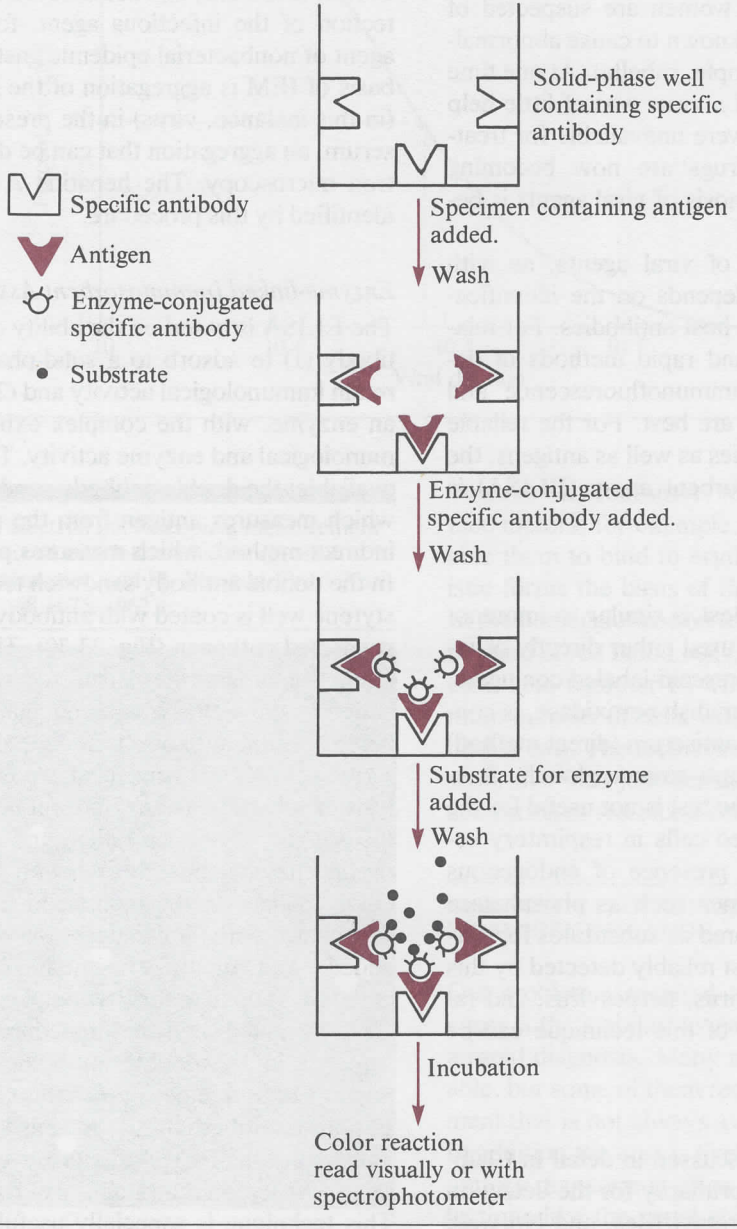
Immunoelectron Microscopy

Immunoelectron microscopy (IEM) is essentially a research tool since most laboratories do not have an electron microscope. In certain diseases, however, it is the only in vitro method available for detection of the infectious agent, for example, the agent of nonbacterial epidemic gastroenteritis. The basis of IEM is aggregation of the infectious agent (in this instance, virus) in the presence of immune serum, an aggregation that can be detected by electron microscopy. The hepatitis A virus was first identified by this procedure.

Enzyme-linked Immunosorbent Assay

The ELISA is based on the ability of antigen or antibody (1) to adsorb to a solid-phase support and retain immunological activity and (2) to be linked to an enzyme, with the complex exhibiting both immunological and enzyme activity. Two methods are available: the double antibody sandwich technique, which measures antigen from the patient, and the indirect method, which measures patient antibody. In the double antibody sandwich technique, a polystyrene well is coated with antibody specific for the suspected pathogen (Fig. 33-30). The clinical specimen containing the suspected viral antigen is added to the well. Next a conjugate of enzyme-labeled specific antibody is added, and a complex is formed. Later enzyme substrate is added. Hydrolysis of substrate by enzyme will be proportional to the amount of antigen bound and can be detected by an enzyme-substrate reaction that produces a color change. In the indirect method, the well is first coated with viral antigen, then patient serum is added, and finally enzyme-labeled anti–human globulin is added to form a complex. Again the substrate is added, and the procedure is virtually the same as in the double antibody sandwich technique. Color change is proportional to the concentration of antibody in the patient's serum. The advantages of the ELISA are its low cost for supplies, its sensitivity, and the stability of the reagents used. This technique is especially useful for hepatitis B

Fig. 33-30. Double antibody sandwich technique used in enzyme-linked immunosorbent assay (ELISA). Antibody is part of the solid-phase well to which is added an aliquot of specimen containing unknown antigen. Enzyme conjugated with antibodies specific for suspected antigen in the specimen is added. An antibody-antigen-enzyme complex is formed. The system is washed to remove nonspecific complexes. A substrate for the enzyme is added, and later the system is washed to remove nonspecific enzyme-substrate complex. Provided the unknown antigen is specific for antibody in the system, an enzyme-substrate complex can be detected. Enzyme-substrate reaction is usually some color reaction that can be detected visually or spectrophotometrically.



virus and rotaviruses and is being considered as a replacement for other techniques for detecting measles, rubella, and herpes viruses.

Neutralization Test

The neutralization test relies on the neutralization of virus by virus-specific antibodies found in the serum. Dilutions of the patient's serum, containing antibodies against the suspected virus, are mixed with aliquots of known virus and inoculated into susceptible cells. The highest dilution of antiserum preventing a cytopathic effect (a morphological change in the cell) as compared to the cytopathic effect produced by a virus-serum mixture free of antibodies is considered the end point. This test, one of the most sensitive and accurate assays, can be performed in cell culture, embryonated eggs, or animals.

Hemagglutination Inhibition Test

In the hemagglutination inhibition (HI) test the patient's serum is first heated or chemically treated to destroy nonspecific inhibitors of the hemagglutination process. The dilutions of the patient's treated serum are prepared in tubes, and to each tube is added a specific dose of the suspected virus. Red blood cells are then added to the mixture and incubated for 1 hour. The HI titer is the highest dilution of serum inhibiting hemagglutination. This dilution is compared to the controls, which contain known antisera. Not all virus particles are capable of hemagglutination and therefore HI may not be used to assay the titer of a patient's serum antibody specific for these viruses.

Gel Diffusion

In the gel diffusion procedure, described in Part IV, Immunology, antigen and antibody are placed in adjacent wells cut into agar on Petri dishes or slides. The reactants diffuse into the agar, and where antigen and antibody meet, precipitation lines develop. The test can be accelerated by combining it with the technique of electrophoresis.

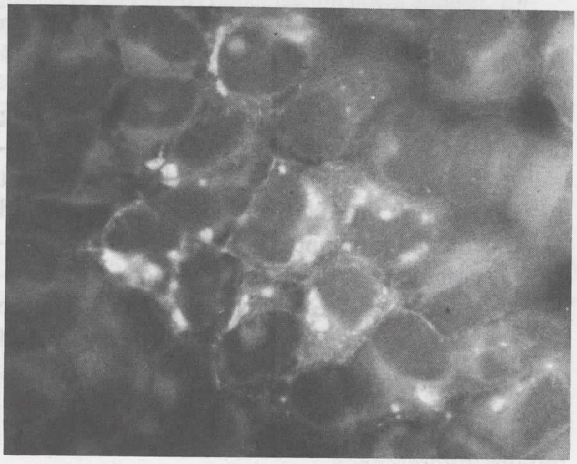
Complement Fixation

The complement fixation test is a frequently used serological test. It is designed to detect antibodies and rises of antibody in paired sera. The basis of the complement fixation test is that virus-antibody complexes bind complement, making the latter unavailable for lysis of sheep red blood cells by hemolysin. The patient's serum is heated to inactivate preformed complement and then is serially diluted. Virus plus complement derived from guinea pig serum is added to each dilution. The mixture is allowed to incubate in the cold to let the complement be fixed. After the incubation period, sheep red blood cells and a hemolysin are added to the mixture. If complement has been fixed to the antigen-antibody complex, the hemolysin will not lyse the red blood cells; if complement has not been fixed by the test antigen and antibody, the red blood cells will be lysed.

Immunofluorescence

In the technique of immunofluorescence either a direct or an indirect approach can be taken to the detection of virus in virus-infected cells. In the direct method viral antigen is exposed to a specific fluorescein-tagged antiserum. If specificity exists between the virus and the antiserum, the complex will be seen to fluoresce when observed microscopically (Fig. 33-31). In the indirect method the virus-specific antiserum is not tagged with the fluorescein dye. Instead, fluorescein is conjugated to anti–human gamma globulin. In this procedure the virus is allowed to bind to the unlabeled virus-specific antiserum. The fluorescein-conjugated anti–human gamma globulin is then added to the mixture, producing a complex of virus, unlabeled antiserum, and fluorescein-labeled anti–human gamma globulin, which fluoresces. The indirect method has the advantage of requiring only a single fluorescein-tagged reagent and does not call for the tagging of every virus-specific antiserum. Fluorescent antibody has been used to detect a number of viruses, but it is primarily used for the rabies virus and the causal agents of hemorrhagic fevers (Lassa fever, Ebola, Marburg, Junin, and Machupo viruses).

Fig. 33-31. Fluorescent focus of Ebola virus in HeLa cells. (From A. L. Truant, *J. Clin. Microbiol.* 18:416, 1983.)



EFFECT OF CHEMICAL AND PHYSICAL AGENTS ON VIRAL ACTIVITY

In Vitro Inactivating Agents

The process whereby a chemical or a physical agent is capable of rendering a virus noninfective is called *inactivation*. Inactivation is caused by an agent's ability to react with the protein, nucleic acid, or lipid components of the virus. The loss of infectivity that results from inactivation can be due to the virus's inability to penetrate the cell, to replicate in the cell, or to express some important genetic function within the cell.

AGENTS THAT REACT WITH VIRAL PROTEINS. Agents that react with proteins are directed against the capsid and include the following:

1. Proteolytic enzymes. The enzyme pronase can inactivate some viruses. The enzymes of the intestinal tract, such as trypsin, chymotrypsin, and pepsin, are for the most part not active against the viral coat protein.
2. Temperature. In some viruses protein denaturation can occur at 37°C; many, however, are resistant to that temperature. Temperatures of 55–65°C for 1 hour will inactivate most viruses, except the polio and serum hepatitis viruses. The inactivation may come about because heat affects the configuration of the capsid protein and prevents uncoating during viral replication or because the protein of the glycoprotein envelope of enveloped viruses is damaged, which prevents the virus from attaching to host cells. The addition of molar concentrations of salts to an aqueous viral suspension can protect most viruses from inactivation at elevated temperatures. Freezing has no effect on viruses, and the freezing process is actually used to preserve them.
3. Phenol. The compound phenol so alters the capsid that it can bring about the release of nucleic acid from within the capsid protein.
4. Radiation. Ultraviolet absorption at wavelengths of 235 nm in the protein is due to the presence of the peptide bond and can result in denaturation. Aromatic amino acids absorb at wavelengths of 280 nm.

AGENTS THAT REACT WITH VIRAL NUCLEIC ACIDS. Many of the agents used to produce viral and bacterial mutants in the laboratory can also be used to inactivate viruses. Inactivation is accomplished either by increasing the dosage of the mutagenic reagent or by increasing the length of time of treatment. The agents that react with viral nucleic acids include the following:

1. Formaldehyde. Aqueous solutions of formaldehyde (formalin) react with amino groups, especially those of the nucleic acid bases in single-stranded molecules. Their effect on the amino groups of the capsid protein is not enough, however, to interfere with the antigenic capacity of the virus. For this reason formalin is used in the preparation of some viral vaccines.
2. Nitrous acid. Nitrous acid deaminates the purine and pyrimidine bases of nucleic acids, a condition that can lead to mistakes in the replication process of the viral DNA.
3. Radiation. Nonionizing ultraviolet light at 260-nm wavelength is strongly absorbed by purine

and pyrimidine bases. Inactivation of viruses by ultraviolet light is due to its effect on the pyrimidine bases. For example, covalent bonds may be produced between adjacent thymine residues in DNA (see Fig. 5-13) or uracil residues in RNA to produce dimers. Dimer formation prevents normal DNA replication. Further, the double bond at carbons 5 and 6 in pyrimidines may become hydrated as a result of ultraviolet treatment, an effect believed to be important in inactivating RNA viruses. X-rays can damage the nucleic acid molecule by causing breaks in the sugar phosphate backbone. The treatment of viral suspensions with x-rays can also lead to the production of free radicals that act as oxidizing agents, attacking the nucleic acid molecule.

4. Photodynamic effect. Photodynamic inactivation is a process in which a dye such as methylene blue or various acridines is added to a viral suspension to interact with the nucleic acid. When this complex is later illuminated with visible light, the virus becomes inactivated.

AGENTS THAT REACT WITH VIRAL LIPIDS. Many viruses contain a lipid envelope that surrounds the capsid. A few agents are capable of dissolving this envelope. They include ether, bile, and anionic detergents such as sodium dodecylsulfate. In addition enzymatic digestion is possible with such enzymes as the phospholipases; however, not all enveloped viruses are affected by them.

Antiviral Chemotherapy

Many people find it difficult to understand why viruses cannot be treated with the same drugs that are effective against bacteria. The antibiotics used against bacteria for the most part inhibit some process that is peculiar or specific to the bacteria. For instance, the antibiotics that inhibit bacterial cell wall formation are of obvious value in therapy because mammalian cells have no cell walls and are thus not affected. For antibiotics that do inhibit both bacterial and host cell metabolism, the concentration of drug required is generally so small that the cells of the body are not adversely affected. The situation with viruses is different because the virus does not have its own metabolic machinery—it relies on the host's metabolic systems. Thus antiviral agents that inhibit viral metabolism also inhibit host cell metabolism. The situation is also complicated by the fact that extremely high concentrations of antiviral agents are required for chemotherapy. Ordinarily, even high concentrations of drug are without effect during the height of the disease. Since the symptoms of most viral diseases do not appear until the viruses have completed their replication cycle, antiviral chemotherapy is often of little value. The most successful approach to viral disease is prophylaxis—vaccines. In the future, chemotherapeutic agents may be found that effectively inhibit viral activity, but the increased use of such agents will raise the problem of drug resistance, a problem not to be taken lightly.

In the search for antiviral agents, scientists hope to find compounds that exploit differences between viral and host metabolism. These differences have centered on viral nucleic acid synthesis and the enzymes with which they are associated. The enzymes involved in viral RNA synthesis and DNA synthesis in poxviruses, for example, are not present in uninfected cells. Only a few drugs have been developed that have proved effective in the treatment of diseases associated with these viruses. The following is not a complete list of such agents; only those that are currently of medical interest are considered.

AMANTADINE HYDROCHLORIDE. Amantadine hydrochloride (α-adamantanamide hydrochloride) inhibits the primary transcription process in the influenza A virus and thus prevents the uncoating process. Amantadine hydrochloride is used for the prevention of influenza A, but it is not a substitute for the influenza vaccine. Maximum effectiveness can be obtained only when the drug is administered at the start of the influenza season and is maintained daily as long as virus exposure is certain (usually 6–8 weeks). The drug is used more extensively in the Soviet Union than in the United States, where it is administered only to high-risk groups. The drug can cause side effects such as diz-

ziness, depression, and drowsiness, particularly in the elderly.

Rimantadine is an analog of amantadine that has been used with considerable success in the prophylaxis of influenza. The drug has been used for years in eastern Europe but only recently has it been studied in the United States. The drug appears to present fewer side effects, particularly for the elderly, than amantadine.

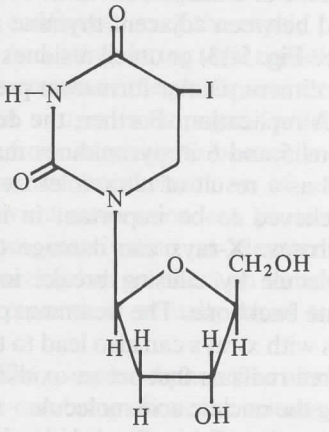
Ribavarin (virazole) is a synthetic nucleoside resembling guanosine that is active against both RNA and DNA viruses. The drug appears to inhibit an early viral replication step that leads to the synthesis of viral nucleic acids. Ribavarin has been used in clinical trials for the treatment of respiratory syncytial virus (RSV) infection. The drug has been shown to be particularly useful in the treatment of infants, in whom RSV infections can be fatal. Ribavarin was shown in 1984 to be capable of inhibiting the in vitro replication of the virus that causes acquired immune deficiency syndrome (AIDS). Experiments in humans were begun in 1985.

METHISAZONE. Methisazone (N-methylisatin-β-thiosemicarbazone) belongs to a group of compounds called *thiosemicarbazones*. This drug specifically inhibits the translation of late mRNA and thus prevents synthesis of viral structural proteins. Methisazone was used as a chemoprophylactic agent against smallpox in areas where the disease was endemic (smallpox was declared eradicated in 1977). It has been used chemotherapeutically in the treatment of complications, such as gangrene and eczema, arising from smallpox vaccination.

RIFAMPIN. Rifampin and other rifamycin derivatives inhibit the multiplication of poxviruses and adenoviruses by interfering with some event in viral morphogenesis. Rifampin also inhibits the RNA-dependent DNA polymerase (reverse transcriptase) of RNA tumor viruses.

RIBONUCLEOSIDES AND DEOXYRIBONUCLEOSIDES. Ribonucleosides and deoxyribonucleosides represent a group of antiviral drugs that have been used with some success in the treatment of herpesvirus-associated diseases. All of these compounds inhibit nucleic acid synthesis and when taken into the cell are phosphorylated and incorporated into viral as well as cellular DNA. Their antiviral effect derives from the fact that viral-coded enzymes used in the biosynthesis of nucleic acid are more affected by the drug than are similar cellular enzymes. *Idoxuridine* (5′-iododeoxyuridine or IDU) is an analogue of thymine (Fig. 33-32) whose use is restricted to local infections, such as the eye infections caused by herpes simplex virus type 1. Because the infected cells of the eye do not divide, only viral DNA incorporates IDU, which inhibits the normal pairing between viral DNA strands.

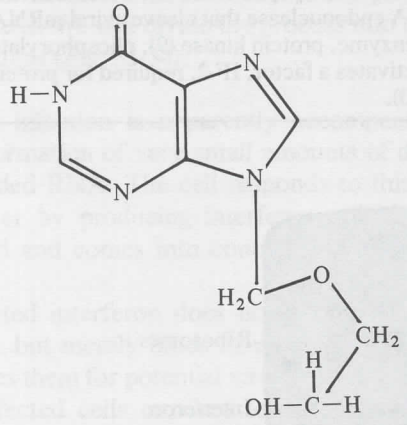
Fig. 33-32. Structure of idoxuridine (IDU).



Vidarabine (adenine arabinoside, ara-A) has a spectrum of activity similar to that of IDU. Vidarabine is effective in the treatment of life-threatening herpesvirus infections. It has been used in the treatment of herpes zoster infections of immunocompromised patients and herpes simplex infections of the brain.

Cytarabine (cytosine arabinoside, ara-C) was used in the treatment of herpes zoster, but clinical trials indicated that the drug was more toxic than had been expected and was less efficacious than vidarabine.

Fig. 33-33. Structure of acyclovir.



ACYCLOVIR. *Acyclovir* (9-[(2-hydroxyethoxy)methyl] guanine) (Fig. 33-33) is an antiviral agent that has been demonstrated to be effective in the treatment of herpes simplex virus infection. Before the drug is active as an antiviral agent it must be monophosphorylated by virus-specific thymidine kinase and then phosphorylated by cellular kinases to a triphosphate derivative. It is the triphosphate derivative that inhibits viral DNA polymerase. Acyclovir-resistant mutants with diminished thymidine kinase activity have been discovered, but these mutants appear to have reduced virulence.

INTERFERON PRODUCTION. In the late 1950s Isaacs and Lindemann discovered interferon during studies on viral interference. They demonstrated that chicken cells infected with influenza virus released a product that protected other chicken cells from infection not only by influenza virus but by a wide variety of other viruses as well. Interferon is a biologically active compound, and concentrations as low as 3×10^{-14} M are sufficient to impair viral replication in cell cultures.

Interferons are a group of glycoproteins of similar sequence homology that are found in most vertebrates including humans. Unless induced, the level of interferon in various organs and tissues is virtually undetectable. In addition to viruses a variety of other products, including endotoxin, specific bacterial species, and polysaccharides, can induce cells to produce interferon. Viruses and double-stranded RNA are the most active inducers of interferon. DNA and RNA viruses during the multiplication cycle produce double-stranded RNA, which is the apparent inducer of interferon in vivo. The mechanism of action of these inducers is not known. Synthetic RNA duplexes of homopolymers such as poly rA: poly rU are potent inducers of interferon. Synthetic double-stranded RNA inducers were initially used to induce interferon in humans but were too toxic when administered systemically. Trials are continuing to find less toxic alternative synthetic inducers.

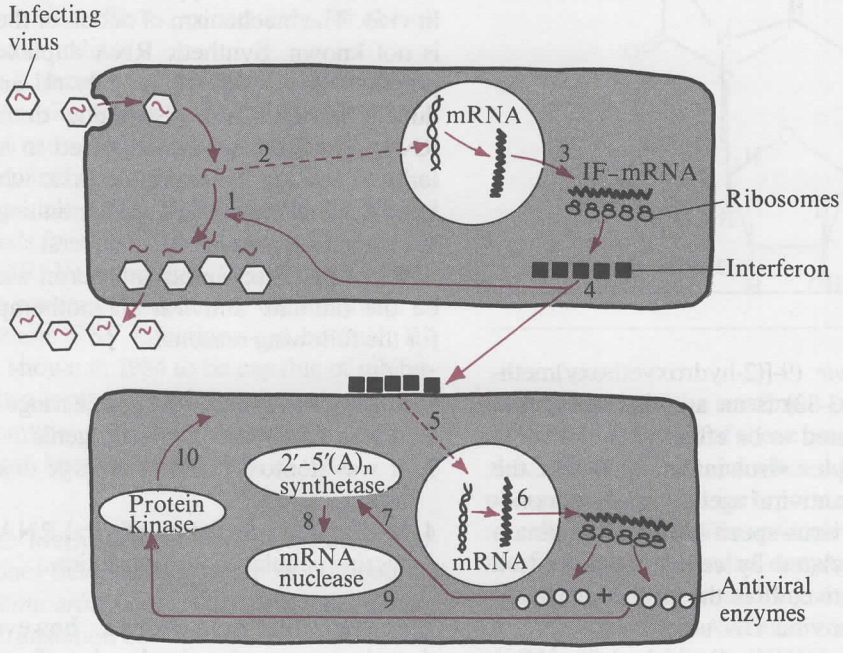
When first discovered, interferon was thought to be the ultimate antiviral chemotherapeutic agent for the following reasons:

1. It was effective against a wide range of viruses.
2. It was almost totally nonantigenic.
3. It was nontoxic, and thus large doses could be administered.
4. It inhibited translation of viral RNA without affecting cellular RNA translation.

Further interferon studies, however, revealed that there were drawbacks. Interferon is species specific as well as tissue specific. Interferon produced in human cultures is more effective against human virus-infected cells than is interferon produced by other animal species infected by the same virus. Thus, to be an effective antiviral agent interferon should be produced from human cell lines. To date, this has been a difficult and expensive process that results in very low yields of interferon, but, as we will see later, this problem has been largely circumvented. Different tissues of the body produce biologically distinguishable interferons. The concentrations needed to affect an antiviral result in human disease are very high. A culture of several million infected cells produce only a few picograms (10^{-12} gm) of interferon; thus it would take many liters of culture to produce only a few micrograms.

Human interferons belong to three different fam-

Fig. 33-34. Proposed mechanism of interferon action. Infecting virus (1) produces new viral particles but also (2) induces cell to produce interferon mRNA (*IF-mRNA*) (3), which is translated into interferon molecules (4). Interferon released by cell may inhibit development of virus in same cell, or it may bind to adjacent cells and (5) induce the cell to produce antiviral proteins (6) such as 2′-5′$(A)_n$ synthetase (7), which activates (8) an mRNA endonuclease that cleaves viral mRNA's. A second enzyme, protein kinase (9), phosphorylates and thus inactivates a factor, IF-2, required for protein synthesis (10).



ilies with little or no cross reactivity: alpha, beta, and gamma. They were at one time designated leukocyte, fibroblast, and immune, respectively. (*Immune* refers to lymphocytes from blood or lymphocyte cell lines that are exposed to antigens to which the cells have been sensitized.) We now know that the type of interferon produced depends on the nature of the producing cell and of the inducer. Alpha and beta interferons can be induced in a variety of cells by agents such as viruses, certain bacteria, and double-stranded RNA, while gamma interferons can be induced in lymphoid cells by mitogens and by antigens to which the cells have been sensitized. Human interferons for clinical trials have been produced by leukocytes induced by Sendai virus. A breakthrough in the technology of interferon came about in 1980 through the use of recombinant DNA technology. This technique has allowed for the synthesis of much larger amounts of interferon with greater purity by bacteria. The recombinant DNA technique involves the following procedures:

1. mRNA isolated from human leukocytes is induced by infecting them with Sendai virus.
2. Leukocyte mRNA is fractionated, and the mRNA with the highest interferon activity is used as a template for synthesis of double-stranded complementary DNA.
3. The double-stranded DNA is inserted into a bacterial plasmid, which is used to transform the bacterium *E. coli*.
4. Transformed *E. coli* produces human interferon.

Many questions remain unanswered as to the mechanism of action of interferon, but enough information is known to present a broad picture of the process. We will divide the process into the following steps (Fig. 33-34):

1. Virus infection is apparently accompanied by the formation of very small amounts of double-stranded RNA. The cell responds to this RNA inducer by producing interferon, which is secreted and comes into contact with uninfected cells.
2. Secreted interferon does not affect viruses directly but merely binds to uninfected cells and primes them for potential viral invasion.
3. Uninfected cells exposed to interferon are induced to engage in protein synthesis and the formation of latent enzymes that do not impair the metabolism of the uninfected cells. One of these enzymes is a polymerase that links together adenosine monophosphate (AMP) residues from adenosine triphosphate (ATP) by means of 2′- to 5′-phosphodiester linkages. The oligonucleotide produced has the structure

   pppA (2′p5′A)*n*

   where $n = 1$ to 10. This enzyme, called 2′-5′$(A)_n$ synthetase, in turn activates another enzyme, an endonuclease, which preferentially cleaves viral mRNA's. A second enzyme induced by interferon is protein kinase. Protein kinase phosphorylates one of the protein factors, called *IF-2*, required for initiation of protein synthesis.
4. Viral maturation is therefore stopped in two ways: cleavage of viral mRNA synthesis and inhibition of viral protein synthesis.

Interferon is still an experimental drug that is being used in a variety of diseases, both viral and nonviral. Interferon has been effective in trials for treatment of varicella, herpes zoster, and hepatitis B and for the prevention of cytomegalovirus-associated infection after renal transplantation. Interferon has been discovered to have several other functions in the cell. Interferons affect cell motility, cell proliferation, and immunological processes such as the antibody response, delayed hypersensitivity, graft rejection, natural killer cell recruitment, and macrophage activation. The discovery of these functions has led to several trials in cancer patients. These studies have revealed that interferon is effective in controlling cancers such as osteogenic sarcoma and breast cancer.

Vaccines

The historic epidemics, such as smallpox and yellow fever, that once decimated large populations are no longer a major threat to modern society. In some underdeveloped nations of the world, outbreaks still occur, but except in extreme cases they are generally curtailed before they involve large masses of people. One of the primary reasons massive epidemics no longer exist is the development and extensive use of vaccines. Vaccines, whose function is to elicit an immune response, have historically been prepared from living or inactivated viruses. As we will discuss later, new techniques are now available for producing vaccines.

Live vaccines are prepared from living avirulent viral strains. That is, the original virulent strain has been attenuated so that the virus is still capable of replicating and stimulating the formation of antibodies but is unable to elicit the major symptoms of the disease. This reduction in virulence is accomplished by repeated passage of the virus in animals and animal tissue culture cells. The cultured virus is then tested for virulence in susceptible animals and, if found to be sufficiently attenuated, is applied to human volunteers. An important advantage of live preparations over inactivated ones is that only a single dose of virus is required to produce a long-lasting immunity. With inactivated vaccines, boosters are generally necessary.

Major problems are associated with live vaccine preparations:

1. Genetic stability of the virus. It is theoretically possible for an avirulent strain to mutate to the virulent form. In the development of a stable strain of virus, a balance must be reached in

which the virus is sufficiently immunogenic yet relatively nonpathogenic.
2. Heat stability. In vaccine preparations a specific number of viral particles is required to ensure maximum immunization. Heat can reduce this number and render a vaccine ineffective. A vaccine preparation should not be left out at room temperature for any length of time and should be stored under refrigeration.
3. Contaminated preparations. Cell cultures used in the production of virus may be contaminated with endogenous animal virus. Simian virus 40 (SV40) was originally discovered in monkey kidney cells being used for polio vaccine production. Many animal viruses such as SV40 can be manipulated in the laboratory and shown to be oncogenic (tumor producing). Conceivably, any animal virus may carry tumor-inducing genes, and their contamination of vaccines and human cells represents a potential threat. Cell lines in which contaminating animal viruses were detected have in most instances been discontinued and replaced with virus-free cultures.

Viable bacteriophages have also been detected in commercially licensed vaccines. Their appearance in vaccines is due to the contamination of fetal bovine serum, which is used as a source of growth factors necessary for the propagation of cells in tissue culture. The fetal serum is often contaminated with bacteria. Filtering of the serum before use removes the bacteria but not the bacteriophages. Although the evidence is only circumstantial, bacteriophages might have the following effects on human cells:

1. Phages have been manipulated in the laboratory to carry genes that human cells lack. The gene-deficient human cells following bacteriophage infection can translate the genetic information carried by the virus into a protein. It is possible that the translation of certain viral information could cause degenerative changes in the cell.

2. It is well known that some strains of bacteria are pathogenic only when they are lysogenized by temperate bacterial viruses. For example, the bacteria that are the causal agents of diphtheria and scarlet fever harbor bacteriophages that carry the information for the production of potentially lethal toxins. Infection of human cells with bacteriophages containing toxin determinants could have serious consequences.

As of this writing no definitive correlation has been observed between bacteriophage contamination of vaccines and increased incidence of infection or degenerative disease. Nevertheless, such contamination must still be considered a problem in virus vaccine production and should be treated as one. Data on the effect of bacteriophages on mammalian cells must await more experimental research by the molecular biologist.

Inactivated vaccines are made from viral preparations that have been treated with formaldehyde, betapropiolactone, or phenol. Only a few inactivated viruses are used in vaccines today, for example, the influenza vaccine. Some of the older vaccines, such as the Salk polio vaccine, have been replaced with live preparations (Sabin vaccine).

One of the original reasons for the use of inactivated vaccines instead of the live preparations was the fear that inoculation with a potentially virulent virus could result in fatal systemic infections. Because of the high standards required by government agencies, this problem as well as others have been largely overcome. Potential problems must never be overlooked, however, and quality control should be maintained and increased wherever possible to ensure the production of safe viral preparations.

Several strategies have been attempted with various virus vaccines to increase their effectiveness: recombination, temperature sensitivity, and subunit utilization.

Recombinant vaccines have been prepared with the influenza virus. This virus contains an RNA that is composed of eight distinct single-stranded segments. Infecting cells with two different influenza A strains creates a recombinant or "hybrid" containing characteristics from both strains.

Temperature-sensitive mutants have practical value especially for viruses that infect the respira-

tory tract. For example, temperature-sensitive mutants are selected that cannot replicate at the higher temperatures of the lower respiratory tract (that is, 37°C) but can replicate in the upper respiratory tract where temperatures are lower (that is, 32–34°C). Thus pulmonary pathological effects can be prevented.

Another approach is to design vaccines not around an infectious agent but around the subunits or antigenic surface components of which the microbial agent is composed. *Subunit vaccines* are ideal because they overcome the problems of contamination and storage and because they are devoid of the myriad antigens that are found with the whole pathogen. The surface components, for example, the pili (fimbriae) of *Neisseria gonorrhoeae* or capsular antigens of *Streptococcus pneumoniae*, have been used for the development of bacterial vaccines. Viruses have specific sites on the capsid or envelope that are subject to neutralization by antibodies. The subunit of the hepatitis B virus is found normally in the serum of chronically infected individuals. These subunits have been purified and are the basis of the currently used vaccine. This type of subunit vaccine is expensive to produce, and specific subunits are not usually present in the serum of the host after infection by other viruses. Other approaches to subunit vaccines are now available because of the development of monoclonal antibodies and recombinant DNA technology. Subunits can be physically removed from the virus after virus cultivation. For example, the hemagglutinin glycoprotein of the influenza virus is prepared in this way for vaccine development. One approach to subunit vaccine development is to isolate and clone the genes for the major antigen. The gene is inserted into a microbial cell (yeast or *E. coli*, for example), and the latter produces the protein antigen. The protein is isolated and crystallized, and its three-dimensional structure is determined. It is not always necessary to isolate the protein antigens. It is easier to isolate the genes responsible for producing the antigens and then determine their amino acid sequences. Once the DNA sequences are known, the corresponding protein sequences can be deduced using the genetic code. This information can be fed into a computer, which will make an educated guess as to which amino acids lie on the antigen's surface. These amino acid sequences can then be synthesized in the laboratory.

Synthetic subunit vaccines are still experimental. One of the problems is that their protection is not long lasting. Carriers that can be linked to the peptides are being developed to improve the length of protection. The future looks very promising for the development of cheap, safe, and stable vaccines for many of the important infectious diseases of humans. This development of subunit vaccines also has important implications in veterinary medicine. Nearly 10 percent of all calves and piglets die from infectious diseases (many of viral origin) in the United States. This figure is nearly 30 percent for Third World countries. If these losses could be curtailed by vaccines, the amount of protein in the world food supply would increase dramatically.

ONCOGENIC VIRUSES AND CANCER

Since cancer has never been shown to be contagious, it is hard to see how this disease could be due to infectious agents. Cancer in humans has usually been thought to be caused only by chemical or physical agents because tumors could be induced in people and animals with these agents. Early in the twentieth century, investigators Shope, Rous, and Bittner, among others, demonstrated that viral agents were responsible for some neoplasms (abnormal growth of tissue) in animals. There was no reason to believe, however, that viruses were involved in human cancer until 1951, when Gross discovered that a mouse leukemia induced by an animal virus was very similar to the leukemia that afflicts humans. Since Gross's discovery scientists the world over have been actively attempting to determine the association, if any, between viruses and human cancer.

Many viruses are capable of infecting animals in such a way that the affected cells are transformed, resulting in the formation of tumors. These tumor-

initiating viruses are called *oncogenic viruses*. In the following discussion it is well to keep in mind that cancer is actually a very rare response to viral infection. The major transforming agents discussed are not naturally occurring and must be manipulated in the laboratory to obtain the desired neoplastic cellular response.

PROPERTIES OF TRANSFORMED CELLS

Both DNA and RNA viruses are able to induce tumors in experimental animals. In each instance the tumor cell is transformed and acquires properties that make it distinct from uninfected cells or infected cells in which tumors are not produced. Depending on the virus and the cell type involved, these transformation properties are quite variable. A common feature of all cancer-causing viruses is that the viral genome becomes either integrated into or intimately associated with the host DNA. Some important properties of transformed cells are as follows:

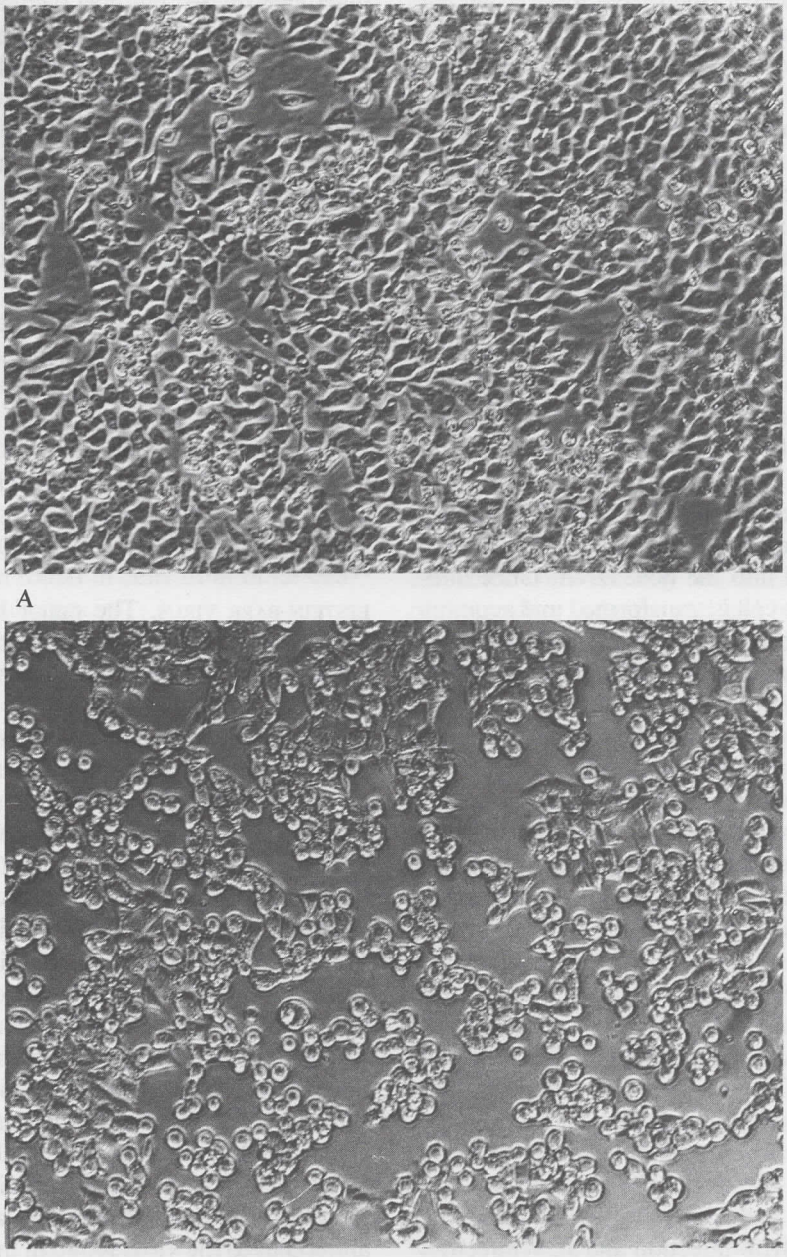
1. As already mentioned, the viral nucleic acid becomes integrated into or associated with the cell DNA, and the host cell does not lyse. This attribute is similar to the property of lysogeny in bacterial cells. Integration can occur with DNA and RNA viruses. How the genome of the RNA virus is integrated is discussed under Oncogenic RNA Viruses.
2. The transformed cells are rounder than normal cells and show irregular patterns of orientation in cell culture (Fig. 33-35).
3. Transformed cells lose the property of contact inhibition and show a loss of growth-inhibiting ability. Changes in the growth patterns of certain transformed cells cause them to form colonies in soft agar. This is a property that permits the investigator to select transformed from untransformed cells.
4. Some transformed cells can produce tumors when injected into susceptible animals. Whether the tumors are benign or malignant is dependent on the transformed cell used and the type of host being challenged.
5. After transformation, a virus-specific antigen appears on the surface of many tumor cells. It is called the *tumor-specific transplantation antigen* (TSTA). Other antigens may also appear on the surface of the cell that makes it recognizable by the host's immune system. These surface antigens may be host or virus specified.
6. Chromosomal abnormalities, including breakage of some chromosomes and duplication of others, appear after cellular transformation.
7. Several changes occur in the cytoplasmic membrane of transformed cells. One of the earliest changes involves the permeability mechanism. Transformed cells are more permeable to metabolites such as simple sugars than are normal cells. Chemical changes in the membrane involving lipid, carbohydrate, and glycoprotein composition may occur. These changes are not uniform but vary depending on the composition of the growth medium and the growth rate of the cells.
8. The agglutinability of cells by certain glycoproteins called *lectins* or *agglutinins* is altered in transformed cells. The sources of lectins include plant seeds (wheat germ, jack bean), snails, and crabs. Transformed cells are agglutinated more readily by lectins because of alteration of the lectin-binding sites.

ONCOGENIC DNA VIRUSES

Polyomavirus and Simian Virus 40

Polyomavirus was originally isolated from a mouse tumor and is endemic in wild and laboratory mouse populations. It is not, however, associated with spontaneous tumors in mice. Simian virus 40 (SV40) was discovered as a contaminant of monkey kidney cell cultures being used in the cultivation of poliovirus for vaccine production. It cannot induce tumors in its own natural host, the monkey. In the laboratory, however, when either polyomavirus or SV40 is inoculated into certain susceptible rodents or human cells in vitro, it can bring about

Fig. 33-35. Transformation of a cell culture by cytomegalovirus. A. Uninfected control. B. Infected culture. Note rounding up of cells. (From J. F. Baskar, S. C. Stanat, and E. Huang, *Infect. Immun.* 40:726, 1983.)



A

B

the transformation of the infected cells. Only when large doses of polyomavirus or SV40 are inoculated into susceptible animals can tumors be induced in vivo, and the tumors formed have a very low capacity to metastasize. Both viruses can cause the lysis of susceptible cells, but their tumorigenic potential is related to their ability to integrate their genomes into the host's DNA.

During SV40 and polyomavirus infection, changes take place in the cell before and during the transformation process. An initial increase in cellular DNA synthesis is followed by the appearance of two virus-induced antigens: the *T antigen* and the *tumor-specific transplantation antigen*. The T antigen is found in the nucleus of the cell; the TSTA, on the cytoplasmic membrane. Some investigators believe that the T antigen is the transformation factor. If the cell is to be transformed and not lysed by the virus, a recombinational event must occur between the viral DNA and the cellular DNA. Between one and eight viral genomes may become integrated into the host DNA. Once integration occurs, the cell is transformed and acquires some of the properties already discussed. Although SV40 and polyomavirus are not very tumorigenic in vivo, they are studied extensively because they provide a model system in which most of the events of tumor formation can be duplicated in vitro. In the in vitro environment the virus-host interaction is not influenced by a host immune system. Much of our present knowledge concerning the biochemistry of oncogenic viral infection is derived from the study of these two viruses.

BK and JC Viruses

Closely related to the polyomaviruses are some human viruses, such as the BK virus (BKV) and the JC virus (JCV). JCV was first discovered in a patient with the demyelinating disease called *progressive multifocal leukoencephalopathy*. BKV was first isolated from the urine of a patient who had received a kidney and ureter transplant 4 months previously. Both BKV infection and JCV infection are widespread, and approximately 80 percent of the human population has antibodies to these viruses. Infection occurs early during childhood, but no clinical condition is found with primary infection. It is believed that these viruses persist in the kidneys after primary infection and that they are reactivated when the host's immune system is impaired. Both viruses are detected in the urine of pregnant women and renal allograft recipients. BKV is highly oncogenic in vitro and can transform a variety of animal cells, but as yet its role in human cancers has not been determined. JCV is also oncogenic for animal cell cultures. JCV when inoculated intracranially into hamsters produces medulloblastomas. Medulloblastomas are the most common brain tumor in children, and as yet JCV is the only viral agent that can produce this type of tumor in animals. JCV is also the only virus capable of producing brain tumors in primates (owl monkey). JCV integrates into cellular DNA in JCV-induced brain tumors; thus there is some circumstantial evidence that this virus may be a human oncogenic virus.

Herpesviruses

EPSTEIN-BARR VIRUS. The major herpesviruses that are known to infect humans are the herpes simplex viruses, the varicella-zoster virus, the cytomegaloviruses, and the Epstein-Barr virus (EBV). Of these only the EBV has been consistently associated with certain human neoplasms. The EBV has an affinity for lymphocytes and is capable of transforming them from end stage cells to proliferating cells with unlimited life spans. The virus was first identified by Epstein and Barr in the early 1960s in cultured lymphoid cells from patients afflicted with *Burkitt's lymphoma*. This rare disease, endemic in certain areas of Africa, is a cancer of the lymphoid system affecting primarily children. As discussed below the EBV has also been implicated in infectious mononucleosis, which is widely regarded as a self-limiting leukemia, and in nasopharyngeal cancer, which has a worldwide distribution.

The first evidence of an association between the EBV and Burkitt's lymphoma was the high level of antibodies to the virus found in the patients' serum. A certain level of EBV antibodies is found in most of the population, particularly in the United States. It is estimated that approximately 80 percent of the

population in the United States have acquired antibody against the virus with no apparent symptoms from infection. EBV infection seems to produce no symptoms in infants, but in some adolescents and young adults it may lead to infectious mononucleosis. Infection is therefore accompanied by the presence of high antibody titers throughout life. The complete EBV particles have never been detected in material obtained at biopsy from any cancer; however, the "footprints" of the virus have not gone unnoticed. The findings that have linked EBV to Burkitt's lymphoma and nasopharyngeal cancer are these:

1. In both Burkitt's lymphoma and nasopharyngeal cancer, EBV-specific membrane antigens and nuclear antigens have been demonstrated. The protein product of the EBV has been detected in the nuclei of cells by immunofluorescent techniques, and it may be similar to the T antigen of polyomavirus and SV40. The EBV nuclear protein has not been found in cells from other cancers or related malignancies.
2. EBV DNA is always found in malignant tissue from nasopharyngeal cancer and in most Burkitt's lymphoma cells. Multiple copies of the EBV genome appear in some of the cells. Some of the DNA may be integrated into the host genome, and some may be free in the cytoplasm.
3. Cancers have been induced in laboratory animals with EBV-containing materials. The cancer produced is almost identical to that occurring in humans and further implicates EBV as a tumorigenic agent.

One of the most puzzling aspects of EBV investigation is the inability of researchers to detect EBV DNA or virus-associated antigens in the malignant cells from the American version of Burkitt's lymphoma.

HERPES SIMPLEX VIRUSES. The herpes simplex viruses (HSV) types 1 and 2 have also been implicated in human cancers. Both viruses produce subclinical infections in humans and can persist in the host in the latent state. HSV 1 is a common human pathogen that affects primarily the mouth (cold sores), while HSV 2 affects primarily the genitalia and is responsible for up to 95 percent of genital herpes infections. HSV 1 and HSV 2 are interchangeable in terms of their site of infection, and both have been observed concomitantly in genital disease. Patients with cancer of the lip or mouth have been shown to carry high titers of antibodies against HSV 1 antigens. Some studies have demonstrated that women with cervical cancer have a higher incidence of HSV 2 antibodies than do those without cervical cancer. A recent study in England, however, has shown that for women attending venereal disease clinics the HSV is not more closely associated with neoplasia than are the other sexually transmitted diseases. So far it has been difficult to detect integrated or free HSV DNA in malignant cervical cells.

MAREK'S DISEASE HERPESVIRUS. Marek's disease herpesvirus (MDHV) causes a malignant lymphoma in chickens. Until recently this disease was a scourge of the poultry industry and had been known to destroy 70 to 80 percent of a housed bird population. For some unknown reason the virus could be found only in the feather follicles of infected birds, from which it was shed in large amounts. Of considerable interest in respect to the human cancer problem is the fact that an effective vaccine has been prepared against this virus. The vaccine was produced from a herpesvirus of turkeys that was immunologically similar to the MDHV. Although it prevents Marek's disease, the vaccine does not prevent replication of MDHV or its spread. Scientists reason that if it can be proved that certain cancers are caused by viruses then it should be possible to make effective vaccines against the cancers.

ONCOGENIC RNA VIRUSES

The mouse leukemia virus found by Gross in 1951 to be transmissible from filtrates of mouse tumors is caused by an RNA virus. Today some RNA viruses are known to cause leukemias and lymphomas in birds, mice, cats, and even certain primates.

The oncogenic RNA viruses belong to the family of viruses called Retroviridae. They are classified as A, B, C, or D according to their appearance in electron micrographs and are infectious for avian or mammalian hosts or both. The most important groups are types B and C. There are two groups of avian RNA tumor viruses: avian sarcoma viruses such as the Rous sarcoma virus (RSV) and avian leukosis viruses (ALV). In nature most RSV can produce tumors only in avian species, in particular the chicken. RSV can infect some mammalian cells, producing tumors in vivo, but they are better known for their ability to transform cells in vitro. When mammalian cells are infected by RSV, no progeny virus is produced. The ALV produce tumors only in chickens.

All RNA tumor viruses have an outer envelope derived from the cell surface membrane and lined with knob-like glycoprotein projections. Within the envelope is a core containing the nucleocapsid. The nucleocapsid contains reverse transcriptase and a diploid RNA genome to which is bonded a transfer RNA (tRNA) molecule. The double-stranded DNA synthesized from the viral RNA template by reverse transcriptase is integrated into the host genome as a provirus. At least one to five proviruses can be integrated at any one time into the cell's genome. One proof that the viral DNA is produced in the cell comes from the experiment in which purified DNA from chicken cells infected with RSV caused the production of RSV in duck cells. Once the provirus is integrated into the cell genome, several pathways are possible:

1. Protected within the genome of the host cell, the provirus may duplicate when the cell DNA duplicates. If the infected cell is a germ cell, the progeny of the host will also carry the provirus; thus vertical transmission may occur.
2. Under certain circumstances the provirus may become activated and excised from the host genome. In this event new virus particles that may infect adjacent cells and tissue can be produced; that is, the transfer is horizontal.
3. Under other circumstances the provirus may also carry information that is oncogenic. If the oncogenic information is translated into specific products it may convert the cell into a tumor cell.

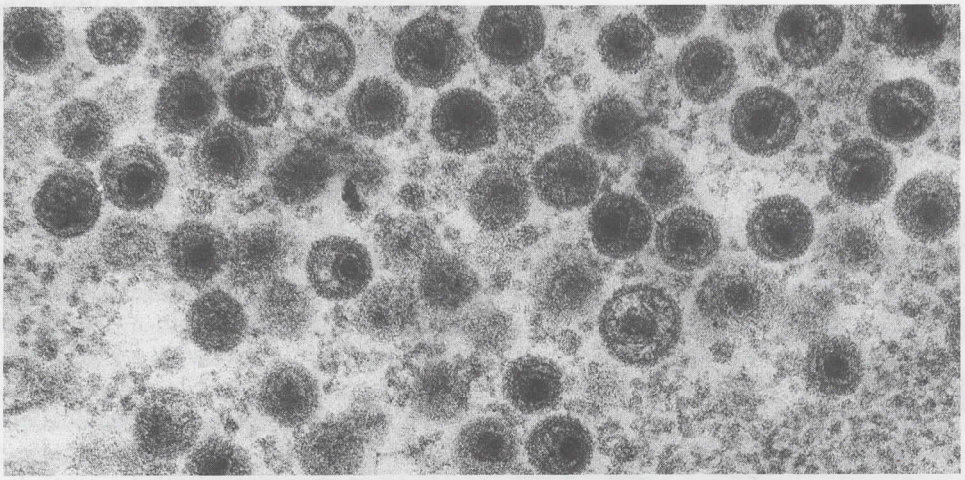
Mammalian sarcoma viruses cannot multiply in their own host and must be rescued by other viruses to produce mature virions. The rescue takes place when the sarcoma virus–infected cell is later infected by an avian leukosis virus. In this doubly infected cell the ALV supplies protein coats that encapsulate the defective sarcoma genome and permits the production of infective sarcoma virus. The sarcoma virus can now infect cells that are in the host range of the ALV.

Oncovirus B

In 1936 Bittner discovered a milk agent as a causal factor in breast cancer in certain strains of mice. This factor was found to be a type B RNA tumor virus. Although capable of reproducing in most mouse cells, the virus, called the *Bittner mouse mammary tumor virus* (MMTV), produces tumors only in mammary glands (Fig. 33-36). Many investigators believe today that a similar virus is involved in the causation of human mammary cancer. No complete viral particles have ever been isolated from neoplastic human mammary tissue. Some circumstantial evidence has implicated a type B RNA virus in mammary tumors:

1. Particles with morphological properties similar to those of type B viruses have been found in human milk samples.
2. A reverse transcriptase that is similar in activity to that of MMTV and not to type C particles has been found in human milk.
3. By means of the DNA produced in vitro from the action of the reverse transcriptase discovered in human milk, it was found that human breast tumor contains RNA homologous to the DNA of the mouse virus. Thus there are base sequences in the nucleic acid of human mammary tumors that are virtually identical to those of MMTV. These RNA base sequences have not been found in other human tumors or in normal human tissue.

Fig. 33-36. Electron micrograph (×70,000) of murine mammary tumor virus type B. (Courtesy G. H. Smith, National Cancer Institute.)



Accumulated evidence shows that milk transmission of human cancer probably does not occur. The increased familial risk of breast cancer appears to be related to an altered hormonal state. Perhaps there is an interaction between hormones and the virus that is responsible for the induction of cancer.

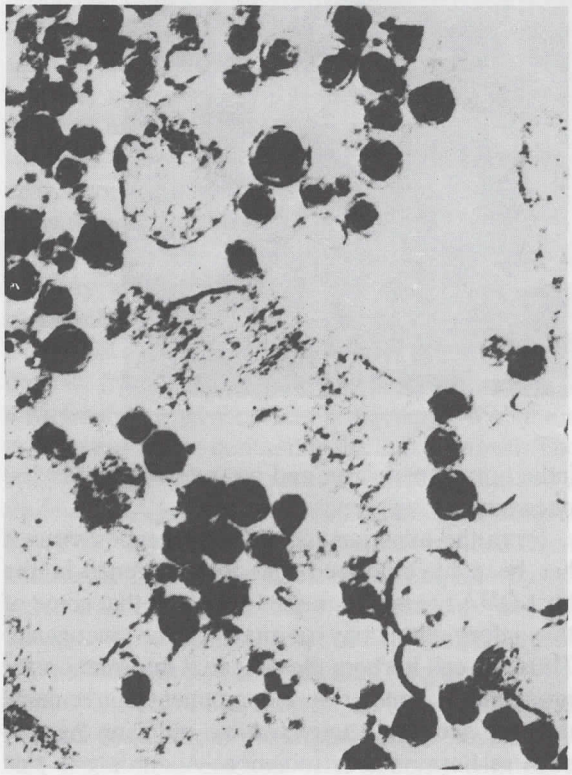
Oncovirus C

Type C retroviruses primarily affect birds and mammals and are divided into three groups: sarcoma viruses, leukemia viruses, and endogenous viruses. The first two groups differ primarily in their in vitro transforming abilities, while the endogenous group shows no pathogenicity in vitro or in vivo. Virus-free cell lines derived from chickens and mice have been shown to produce and secrete type C virus particles either spontaneously or after treatment with chemical or physical agents. These viruses are said to be endogenous type C viruses and are believed to be transmitted vertically from one generation to the next in the gametes. DNA sequences homologous to sequences in the RNA genome of known type C viruses have been shown to exist in the somatic cell DNA of normal tissue from chickens. These nucleotide sequences in the genome of the virus appear to be responsible for the induction of neoplasia and are referred to as viral oncogenes or virogenes.

From the experiments with endogenous virus it has been hypothesized that the virogenes in the host DNA are in a repressed state and that some of this information may or may not be oncogenic. Thus the cell harbors the potential information for producing a transforming component but remains unexpressed until activated by inducing agents, such as hormones or radiation. According to this theory, called the oncogene theory by Hubener and Todaro, the oncogene and virogene information of endogenous viruses is an intrinsic part of the natural makeup of all vertebrate cells. These genes have therefore evolved as cellular entities, as have the various species of animals. Recent developments, as explained in the next section (Cellular Oncogenes and Carcinogenesis), have to some degree clarified the oncogene theory.

A direct role for a virus in human cancer was established by Robert Gallo in 1983 and 1984. Gallo and his co-workers isolated an RNA tumor virus from patients with T-cell cancer. They called the virus human T-cell leukemia virus (HTLV-1) (Fig. 33-37). HTLV-1 infects only T cells and not other kinds of cells in cancer patients. The isolated virus

Fig. 33-37. Thin-section electron micrograph of T cells positive for human T cell leukemia/lymphoma virus. Many mature type C particles are found in the extracellular space. (From W. A. Blattner, et al., *J. Infect. Dis.* 143:406, 1983.)



is also capable of transforming healthy laboratory cells into malignant ones. The virus is endemic in the Caribbean and Africa but cases appear sporadically in the southeastern United States, South and Central America, and Japan. The virus is believed to be spread through mother's milk, sexual relations, and possibly through contact with contaminated blood. HTLV-1 or a very closely related RNA virus has been found in African and Japanese monkeys and it is suspected that the animal virus may have "jumped" to humans within the past two hundred years. HTLV-1 is related to the virus HTLV-111, which Gallo believes to be the cause of AIDS (see Chapter 17).

CELLULAR ONCOGENES AND CARCINOGENESIS

Experiments begun as early as 1960 indicated that the transforming principle is associated with DNA and that the information for being a tumor cell can be transferred from one cell to another. Mammalian cells treated with a chemical carcinogen can be transformed, and the information for transformation is carried in the DNA of the mammalian cells. The DNA from different spontaneous tumors (bladder, colon, and lung carcinomas and others) can transform mouse fibroblasts. The transforming activity is associated with a discrete segment of DNA that hybridizes with a related DNA sequence in the normal genome. In other words, the oncogene represents an altered version of a normal gene. The normal gene that is capable of conversion to an oncogene is called a *proto-oncogene*. It is clear that proto-oncogenes have been conserved in the mammalian genome and that they probably play some role in normal cellular metabolism.

The oncogenes of human tumors are closely related to oncogenes of retroviruses. Two dozen proto-oncogenes have been discovered through the use of retroviruses. Retroviruses can infect a cell and pick up proto-oncogenes and transduce them from the mammalian cell. Somehow the transduced proto-oncogene transforms the cells that the virus happens to infect. Each of the 24 proto-oncogenes so far discovered has been associated with the oncogene of a particular retrovirus. For example, the *myc* proto-oncogene is associated with four distinct avian retroviruses, while the *sis* gene is associated with simian sarcoma viruses. Proto-oncogenes therefore may be activated in two ways: They may become associated with retroviruses, or they may become altered via mutational events that rely on nonviral mechanisms. At least 18 of the different cellular genes have been activated into oncogenes by various retroviruses. A few altered cellular genes have been detected in several types of human and mouse tumors of nonviral causation. Using recombinant DNA techniques it has been shown that a mutation involving a single base pair may be responsible for converting the benign

proto-oncogene *ras* into an oncogene. The mutation results in the synthesis of an altered protein, but the exact function of the protein is not known. The proteins specified by the proto-oncogene are a heterogeneous group that can be activated in a variety of other ways. One activation mechanism that has received considerable attention is called *oncogene amplification*. Amplification of oncogenes has been detected in neuroblastoma cells in which several copies of *myc* proto-oncogenes have been found. Several lines of human lung cancer cells have also been shown to contain extra copies of *myc* genes. Investigators have found that increased expression accompanies gene amplification. This increased gene expression may be what is actually involved in the development of some cancer; however, other mechanisms may also exist. The functional significance of proteins specified by proto-oncogenes has been examined in only a few instances. The *sis* oncogene of simian sarcoma virus has been demonstrated to be homologous to the cellular *sis* gene. The cellular *sis* gene appears to code for platelet-derived growth factor. Evidence is accumulating that the proto-oncogenes are involved in cellular metabolism. Work with Rous sarcoma viruses indicates that the transforming principle may be a kinase enzyme that transfers phosphate groups from ATP to protein acceptors. Such enzymes presumably affect a wide variety of cellular activities including cell division.

VIRAL CLASSIFICATION

Viral classification has been approached in many ways over the past years. With every major advancement in our understanding of viruses, new classification schemes have evolved, centered on (1) symptoms of the viral disease, (2) method of transmission of the virus, (3) symmetry of the viral capsid, or (4) tissue or organ affected by the virus. One of the most useful means of separating the viruses is based first and foremost on the type of nucleic acid carried by the virus. Further separation is based on capsid symmetry and the presence or absence of an envelope. These and other viral properties are summarized in Tables 33-5 and 33-6.

SUMMARY

Viruses are subcellular obligate parasites that consist of a nucleic acid core and a protein coat. They range in size from 25 nm to 300 nm. The protein coat is made up of subunits called capsomeres. The capsid coat may be icosahedral (cubical) or helical (rod shaped), but some viruses have a mixture of the two. Several animal viruses possess a lipid envelope in which are embedded glycoproteins called spikes or peplomers. The viral genome may be DNA or RNA and is either single stranded or double stranded.

Viruses can cause lysis of the cell, or the viral nucleic acid may form a benign relationship with its host by integrating into the host genome. Bacterial viruses that integrate into their host create a condition called lysogeny, while in animal viruses the same condition is called transformation. Transformation alters various properties of the infected cell, but lysogeny alters the phenotype of the bacterial cell only occasionally. The attraction between virus and host involves host and viral receptors. During phage infection only the nucleic acid enters the cell, but in animal viral infections the entire viral particle may enter the cytoplasm of the cell. The virus matures in the host, and viral nucleic acid and protein molecules are synthesized and assembled to produce new viral progeny. Maturation may take place in the cytoplasm or nucleus of mammalian infected cells depending on the virus type. After maturation virus is released from the cell either by lysis of the cell or, as in some mammalian infected cells, by slow release from the infected cell with no lysis. Enveloped viruses obtain their lipid coat as they penetrate through the nuclear or cytoplasmic membrane.

During the bacterial infection process the phage head may encase bacterial genes and transfer them

Table 33-5. Classification of DNA-containing Viruses of Vertebrates

Capsid symmetry	Cubic						Complex
Virion: naked or enveloped	Naked			Enveloped			Complex
Reaction to ether	Resistant			Sensitive			Resistant
Diameter[a] of virion (nm)	18–26	45–55	70–90	100		130–300	230 × 300
Virus family	Parvoviridae	Papovaviridae	Adenoviridae	Herpesviridae		Iridoviridae	Poxviridae
Important genera	Parvovirus	Papovavirus	Mastadenovirus	Alphaherpesvirinae[b], Betaherpesvirinae[b], Gammaherpesvirinae[b]		—	Orthopoxvirus
Human diseases caused by viral family members	Gastroenteritis	Warts	Respiratory infections	Herpes, chickenpox (varicella), shingles (zoster)		—	Smallpox

Table 33-6. Classification of RNA-containing Viruses of Vertebrates

Capsid symmetry	Cubic					Helical			
Virion: naked or enveloped	Naked			Enveloped		Enveloped			
Reaction to ether	Resistant			Sensitive		Sensitive			
Diameter of virion (nm)	24–30	35–39	60–80	40–70	80–120	150–300		60–80	
Virus family	Picornaviridae	Caliciviridae	Reoviridae	Togaviridae	Orthomyxoviridae	Paramyxoviridae		Rhadboviridae	
Important genera	Enterovirus, rhinovirus	Calicivirus	Reovirus, orbivirus, rotavirus	Alphavirus, flavivirus, rubivirus	Influenza virus	Paramyxovirus, pneumovirus, morbillivirus		Lyssavirus	
Human diseases caused by virus family members	Poliomyelitis, gastroenteritis, meningitis		Tick fever, gastroenteritis	Yellow fever, encephalitis, rubella	Influenza	Parainfluenza, mumps, measles		Rabies	

Capsid symmetry	Uncertain				
Virion: naked or enveloped	Enveloped				
Reaction to ether	Sensitive				
Diameter of virion (nm)	100	50–300	80–130	100	
Virus family	Retroviridae	Arenaviridae	Coronaviridae	Bunyaviridae	
Important genera	Type B and C oncovirus	Arenavirus	Coronavirus	Bunyavirus	
Human diseases caused by virus family members	Possibly human tumors	Lassa fever, South American hemorrhagic fevers	Respiratory infection	Encephalitis	

to other bacteria—a process called transduction. Transduction may involve any bacterial gene (generalized transduction) or only specific bacterial genes (restricted transduction).

Animal viruses may be cultivated on the membranes of embryonated eggs or they may be grown in vitro on cultures of animal cells grown on plastic or glass dishes. Only on rare occasions is it necessary to use whole animals for virus cultivation. During growth in the whole animal or in cell culture a variety of changes can occur in the cell. These changes include (1) cytopathic effects—lysis of the cell; (2) the formation of localized areas of host and viral macromolecules called inclusion bodies; (3) fusion of adjacent infected cells; (4) changes in surface antigens; (5) production of an antiviral protein called interferon; and (6) persistent infections, in which all of the host cells are not lysed but instead viral DNA becomes intimately associated with host DNA, bringing about cell transformation and altered cellular growth properties. Persistent infections may be latent, chronic, or slow.

Viable animal virus can be measured by means of plaque assays, pock formation, focus formation, or by serial dilution end point. Total virus can be assayed in a sample by direct measurement using electron micrography or indirectly by the hemagglutination test.

Laboratory diagnosis of viral infection is sometimes impractical as well as expensive, but for some diseases diagnosis is especially important. Most tests involve immunological procedures: immunoperoxidase, radioimmunoassay, immunoelectron microscopy, enzyme-linked immunosorbent assay (ELISA), neutralization, hemagglutination inhibition, gel diffusion, complement fixation, and immunofluorescence.

A variety of chemical and physical agents can inactivate viruses. Agents that interact with viral proteins are proteolytic enzymes, temperature, phenol, and radiation. Agents that react with viral nucleic acids are formaldehyde, nitrous acid, radiation, and the combination of visible light and certain dyes (photodynamic inactivation). Bile salts and other lipid solvents inactivate viruses by interacting with lipid envelopes.

Antiviral agents are few because most substances that affect viral processes affect cellular metabolic processes as well. The available drugs include amantadine hydrochloride, methisazone, rifampin, various ribonucleosides and deoxyribonucleosides, and acyclovir. The natural antiviral agent interferon has now been produced in bacterial cells using recombinant DNA technology. Human interferon is being used in clinical trials for some viral diseases and, because of its effect on certain cellular processes, is also being used in controlling certain human cancers. Viral vaccines are prepared primarily from living attenuated or inactivated viruses. Both types have certain disadvantages. Attenuated vaccines are preferred over killed preparations because living viruses serve as a constant source of antigen and thus provide an elevated primary immune response. The ideal vaccines are those consisting of viral subunits and not the whole virus. The subunits or antigenic components of the virus are now being sequenced with the purpose of producing synthetic vaccines in the laboratory.

Oncogenic viruses cause tumors in many animals, and some are believed to be associated with human tumors. Both DNA and RNA viruses can be oncogenic. Tumor formation is associated with the property of viral transformation. The most convincing evidence for tumor formation by viruses comes from the retroviruses, which are RNA viruses. The oncogenes of human tumors have been identified and are strikingly similar to the virogenes carried by retroviruses. Thus mammalian cells possess genes with normal function that can be induced to become oncogenic; these are called proto-oncogenes. It is believed that retroviruses infect a cell, pick up proto-oncogenes, transduce them to other cells, and transform them. The overexpression of these proto-oncogenes is believed to lead to tumor formation.

QUESTIONS FOR STUDY

1. What are some of the differences between animal and bacterial viral nucleic acids?
2. How does an animal virus obtain its envelope, and of what is it composed?
3. Compare and contrast lysogeny and transformation.
4. Describe the various ways in which an animal virus interacts with the cytoplasmic membrane and enters the cytoplasm of the host.
5. What is the relationship of the terms *lysogeny, lysogenic*, and *temperate virus*?
6. What is meant by *lysogenic conversion*? Give some examples.
7. What is meant by *transduction*? What is the difference between generalized and restricted transduction?
8. Do all viruses carry enzymes? If not why not? What enzymes are carried by some viruses?
9. Which viruses carry the enzyme reverse transcriptase? How does this enzyme operate?
10. What is the function of the HN and F proteins of the paramyxoviruses?
11. What is meant by positive-strand and negative-strand RNA replication?
12. What is meant by contact inhibition during mammalian cell growth?
13. What are the effects of viral infection on the host cell? How are they identified?
14. Why is the term *immunological tolerance* associated with persistent infections?
15. Differentiate between latent, chronic, and slow viral disease.
16. What is the difference between plaques, pocks, and foci in infected animal cells?
17. What is the general mechanism for inhibiting viruses using the agent acyclovir?
18. If interferon is so biologically active at low concentrations why are there problems with its use?
19. Explain in some detail the mechanism of action of interferon.
20. What are the advantages and disadvantages of inactivated and live attenuated vaccines?
21. What is meant by subunit vaccines? How are they prepared? What are their advantages over other vaccines?
22. How does viral transformation affect mammalian cellular growth?
23. What is the relationship of the Epstein-Barr virus to human cancer?
24. What is meant by *proto-oncogenes*? Where are they found?
25. How is oncogene amplification related to cancer in humans?

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34. SELECTED VIRAL DISEASES

OBJECTIVES

To make a list of the principal RNA and DNA viruses

To list the various groups of herpesviruses and the types of infections with which they are associated

To make a list of the viral diseases for which a vaccine is now available

To list those viruses associated with infections of the respiratory tract, digestive system, skin and eyes, cardiovascular and lymphatic system, genitourinary system, and wounds and bites

To understand the concepts of antigenic shift and antigenic drift as they apply to the influenza virus

To describe some of the side effects associated with vaccination for smallpox, rubella, and influenza

To differentiate among hepatitis B, hepatitis A, and non-A, non-B hepatitis as to method of transmission, pathogenesis, laboratory diagnosis, treatment, and prevention

To briefly describe zoster, subacute sclerosing panencephalitis, Reye's syndrome, and Guillain-Barré syndrome

To describe the slow virus diseases

- DNA Viruses and Associated Diseases
  - The Poxviruses
    - Smallpox (Variola)
      - Pathogenesis
      - Epidemiology
      - Laboratory Diagnosis
      - Treatment
      - Immunity
      - Prevention
        - Complications of Vaccination
  - The Herpesviruses
    - Cytomegalovirus
      - Pathogenesis
        - Infection in the Newborn
        - Infection in the Renal Transplant Patient
      - Epidemiology
      - Laboratory Diagnosis
      - Prevention and Treatment
    - Varicella-Zoster Virus (Chickenpox and Shingles)
      - Pathogenesis
        - Varicella
        - Zoster
      - Epidemiology
      - Laboratory Diagnosis
      - Immunity
      - Prevention and Treatment
    - Herpes Simplex Virus
      - Pathogenesis
        - Type 1 Infections
        - Type 2 Infections
        - Other Manifestations
      - Epidemiology
      - Laboratory Diagnosis
      - Prevention and Treatment
    - Epstein-Barr Virus and Infectious Mononucleosis
  - The Adenoviruses
    - Pathogenesis
    - Epidemiology
    - Laboratory Diagnosis
    - Prevention and Treatment
  - The Papillomaviruses
  - The Parvoviruses
    - Norwalk Agent
    - Parvovirus B19
- RNA Viruses and Associated Diseases
  - Measles
    - Pathogenesis
    - Epidemiology
    - Laboratory Diagnosis
    - Prevention and Treatment
  - Mumps (Epidemic Parotitis)
    - Pathogenesis
    - Epidemiology
    - Laboratory Diagnosis
    - Prevention and Treatment
  - Rubella
    - Pathogenesis
      - Postnatal Rubella Infection
      - Congenital Rubella Infection
    - Epidemiology
    - Laboratory Diagnosis
    - Immunity
    - Prevention and Treatment
  - Influenza
    - Classification
    - Pathogenesis
    - Epidemiology
    - Laboratory Diagnosis
    - Immunity
    - Prevention and Treatment
  - Poliomyelitis
    - Pathogenesis
    - Epidemiology
    - Laboratory Diagnosis
    - Prevention and Treatment
  - Rabies
    - Pathogenesis
    - Epidemiology
    - Laboratory Diagnosis
    - Immunity
    - Prevention and Treatment
  - Yellow Fever
    - Pathogenesis
    - Epidemiology
    - Laboratory Diagnosis
    - Prevention and Treatment

Viral Encephalitis
Rhinoviruses and the Common Cold
Enteroviruses
Pathogenesis
Treatment
Parainfluenza Viruses and Respiratory Syncytial Virus
Parainfluenza Viruses
Respiratory Syncytial Virus
The Marburg and Ebola Viruses
The Arenaviruses
Rotaviruses
Slow Virus Diseases
Kuru
Creutzfeldt-Jacob Disease
Subacute Sclerosing Panencephalitis
Progressive Multifocal Leukoencephalopathy
The Hepatitis Viruses
General Characteristics
Hepatitis A Virus
Hepatitis B Virus
Non-A, Non-B Virus
Pathogenesis
Epidemiology
Hepatitis A
Hepatitis B
Non-A, Non-B Hepatitis
Laboratory Diagnosis
Immunity
Hepatitis A
Hepatitis B
Non-A, Non-B Hepatitis
Prevention and Treatment
Hepatitis A Prevention
Hepatitis B Prevention
Non-A, Non-B Hepatitis Prevention

DNA VIRUSES AND ASSOCIATED DISEASES

THE POXVIRUSES

The poxviruses are the largest of the animal viruses and are capable of causing skin lesions in a variety of animals, including humans. Some of the poxviruses are specific human pathogens, while others found primarily in animals may secondarily infect humans.

The poxviruses belong to the family Poxviridae and are divided into subgenera that cause various diseases. The most important subgenus, called *vaccinia*, contains the species that cause vaccinia, cowpox, ectromelia, monkeypox, and variola. All of these share some serological cross reactivity. Variola virus infects only humans and monkeys, while vaccinia virus infects many species of animals and is used in the production of smallpox vaccine. The origin of the vaccinia virus is unknown. It may be derived from Edward Jenner's original strain of cowpox, or it may be a combination of cowpox and variola viruses.

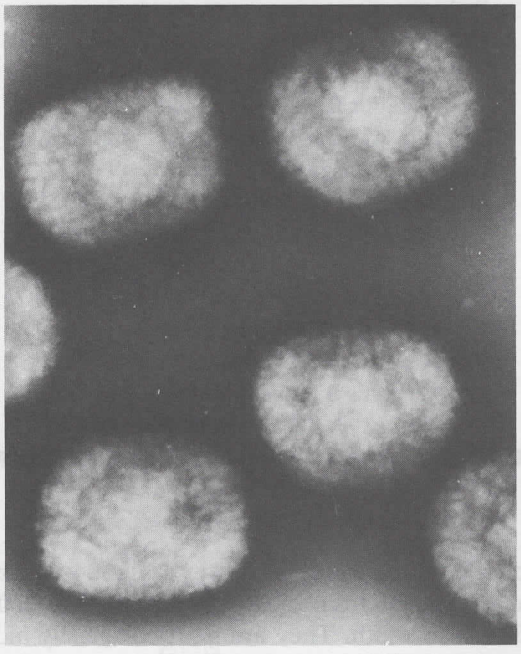
The poxviruses contain double-stranded DNA and are in the shape of a brick (Fig. 34-1). They range in length from 250 to 300 nm and from 200 to 250 nm in width. They are morphologically indistinguishable from one another.

Smallpox (Variola)

Variola, the human infection, is divided clinically into three types based on the severity of the disease: *Variola major*, the most severe form, is found mainly in Asia; *variola minor* (alastrim) is found in South America and Africa; and *variola intermediate* is found in East Africa and Indonesia. The mortality in variola major may exceed 50 percent. Variola minor, the mild disease, has a mortality of less than 1 percent.

PATHOGENESIS. The incubation period for smallpox is 1–12 days before the appearance of symptoms, which include fever, headache, and loss of appetite. In some patients a rash precedes the appearance of symptoms. As the initial fever subsides, there is an eruptive stage in which lesions are seen to progress from macules to papules to vesicles and finally pustules (Fig. 34-2). Fever of varying sever-

Fig. 34-1. Electron micrograph of smallpox (vaccinia) virus. (Courtesy R. C. Williams, Virus Laboratory, University of California, Berkeley.)



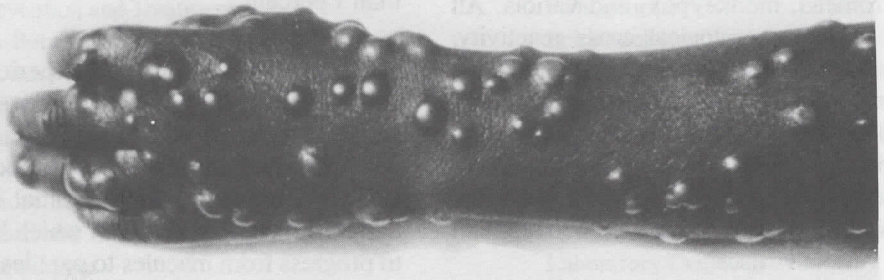
ity accompanies the eruptive stage until the vesicles begin to heal. The smallpox lesions are all in the same stage of development. Crusts form on them after about 10 days and then fall off 2–4 weeks after the onset of the disease. Scarring on the face and arms is common. In the most severe cases the lesions are confluent and become hemorrhagic. There is bleeding from the orifices of the body, followed by shock, coma, and death. In variola minor the symptoms are milder and the course of the disease is shortened.

During the incubation period the virus passes from the upper respiratory tract into regional lymph nodes, where it multiplies before entering the bloodstream. In this primary viremic stage a fever is in evidence. The virus then localizes in the cells of the reticuloendothelial system and begins a second round of multiplication, which causes a more serious secondary viremia that terminates the incubation period. Viremia results in infection of all tissues. Lesions can be found primarily in spleen, liver, lung, testes, and other organs. The virus invades the skin, producing the typical smallpox lesions. Epithelial cells of the infected skin contain intracytoplasmic inclusion bodies called *Guarnieri's bodies*, which can be identified microscopically.

EPIDEMIOLOGY. Smallpox is transferred directly from person to person, although contaminated fomites may also serve as indirect sources of infection. Most infections occur by inhalation of contaminated material. The disease is infectious only after the appearance of clinical symptoms and not during the incubation period. At the onset of symptoms viruses can be found in large numbers in the saliva and later in vesicles.

Vaccination procedures in the United States have resulted in a record of no cases of smallpox

Fig. 34-2. Smallpox pustules on the sixth day of the rash. (Courtesy S. O. Foster.)



since 1949. In 1967 the World Health Assembly in Geneva approved a program to eradicate smallpox from the world within 10 years. At that time smallpox was endemic in 30 countries. Developed countries where smallpox had been wiped out were still at risk of infection via importation. The mass vaccination campaign was aided by the availability of lyophilized (freeze-dried) heat-stable vaccine, the jet gun for administering vaccine, and the lack of a carrier state for the smallpox virus. By 1970, 80 percent of the population in which smallpox had been endemic had been vaccinated. Later mass immunization was not needed, and emphasis was placed on surveillance. Ethiopia was the last bastion of smallpox, but as of the summer of 1976 it appeared that the eradication program had been totally successful. In 1977 smallpox was declared eradicated. Smallpox virus is still stored in some laboratories, and the threat of epidemics still exists. Cases of laboratory-associated smallpox have occurred. Monkeypox virus, closely related to smallpox virus, has caused 21 human infections in Africa. Monkeypox is similar to smallpox but is not as transmissible.

LABORATORY DIAGNOSIS. Presumptive evidence of smallpox infection is based on clinical and epidemiological information. In areas where smallpox is no longer endemic, the clinical symptoms may be confused with those of other viral diseases, and laboratory diagnosis is therefore important.

A rapid presumptive diagnosis of smallpox can be made by direct demonstration of the virus in vesicular material examined by electron microscopy. Inclusion bodies in infected epithelial cells of the vesicles may also be demonstrated by light microscopy.

Variola antigen from vesicular fluid can be detected by the agar gel precipitation test. In this procedure antivaccinia rabbit serum is used as the testing reagent.

Fertile hens' eggs can be used for isolation and identification of variola and vaccinia. For this purpose the chorioallantoic membrane is inoculated at 35°C. Several days later the membrane is peeled from the egg and examined for characteristic variola or vaccinia pock morphology.

Because of the difficulties sometimes encountered with infection in embryonated eggs, tissue culture is a suitable alternative. The tissue cultures used are rhesus monkey kidney cells, infant fibroblasts, or Vero cells. Specimens from vesicular fluid are inoculated into tissue culture suspensions containing bacterial inhibitors. The cells are examined daily for characteristic cytopathic effects.

Serological techniques are generally not employed in diagnosis.

TREATMENT. Vaccinia immune globulin (VIG) prepared from recently vaccinated armed services personnel is used in the treatment of vaccination complications—*vaccinia necrosum* and *eczema vaccinatum*—and for prevention of encephalitis (see under Complications of Vaccination). VIG reduces the severity of infection and is recommended for persons who have been recently exposed to an active case of smallpox. VIG given concurrently with vaccine for those who must be revaccinated greatly reduces the incidence of postvaccination complications.

The drug methisazone has been used successfully to treat vaccinal complications. It has greatly reduced the mortality of vaccinia necrosum. Methisazone is not effective in the treatment of established cases of variola.

Idoxuridine (IDU) is useful for the topical treatment of accidental vaccinia infections of the eye.

IMMUNITY. Maternal antibodies to variola are present in the infant at birth but provide protection for only 3–6 months. The immunity acquired from an active infection is lifelong. The immunity that develops from vaccination is approximately 3–5 years. After this period smallpox infection may occur, but its severity is usually reduced.

PREVENTION. Vaccinia virus is used for vaccination against smallpox. The live vaccine is prepared in the skin of sheep or calves, harvested, and treated with phenol to prevent bacterial contamination. The vaccine is either lyophilized or stabilized with glycerol and stored at −10°C. The lyophilized vac-

cine is used primarily in warmer climates, where viral vaccine stability is a major problem. Reconstituted virus remains stable only a few days under refrigeration. This problem has been circumvented by the development of a trifurcated needle precoated with freeze-dried vaccine. The vaccine does not require rehydration and is stable at nonrefrigerated temperatures for a considerable length of time.

Since 1977 when smallpox was declared eradicated as an endemic disease, the policy for vaccination has changed. Only the following are considered for vaccination:

1. Laboratory workers who work with the poxviruses (smallpox, vaccinia, monkeypox).
2. Military personnel. There has been a concern among the military that variola might be used as a biological weapon. Many other countries have discontinued this vaccination policy since it involves risks both for those vaccinated and for their contacts.
3. Those who might be early contacts of someone with a known case of smallpox. If vaccination in children is required, it should be carried out at ages 1 and 2 years or over to reduce the incidence of vaccinal complications.
4. Those traveling to certain countries where vaccination is a prerequisite for entry. Some countries in Africa and Asia make this condition.

Smallpox vaccine is usually applied to the upper arm by either multiple puncture, multiple pressure, jet injector, or scratch method. After vaccination it is extremely important that excess vaccine be removed from the skin. This procedure helps prevent self-inoculation by fingers into the areas such as the eyes.

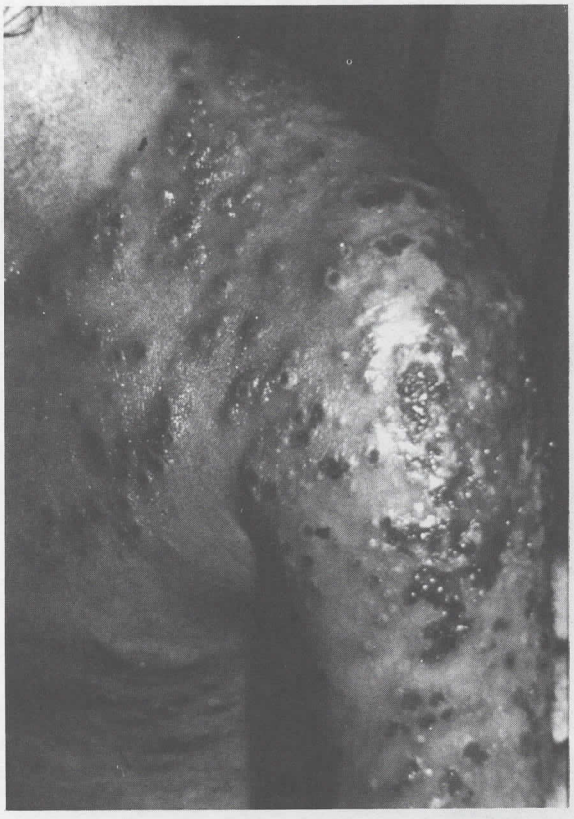
In 1972 the World Health Organization Expert Committee on Smallpox Vaccination recommended the following interpretations of vaccination:

1. Primary take. Primary vaccination is successful when a vesicle develops in 3–5 days and becomes pustular 2–3 days later. A scab is formed and falls off in 2–3 weeks, leaving a scar.
2. Revaccination. Revaccination is successful if a vesicular lesion appears surrounding a previous scab or ulcer. Such successful revaccinations are called major reactions. All other reactions to revaccination are termed equivocal and probably represent an allergic reaction in which a papule appears and rapidly fades without further development. Under these circumstances the vaccination should be repeated at a different site.

Complications of Vaccination. Vaccination may have complications. It is estimated that one complication per thousand and one death per million follow vaccination and that one-half of these are from self-inoculation. Despite the ease with which the virus can be spread after vaccination and the serious complications that can ensue, the vaccine has been misused by some physicians. Smallpox vaccine has been used by some physicians as a treatment for herpes, both oral and genital. In 1983 the only laboratory distributing vaccine discontinued general distribution. Vaccination complications are highest among infants. Possible complications include

1. Postvaccinal encephalitis. Patients with postvaccinal encephalitis have a high fever often followed by convulsions and death. The mortality is often as high as 40 percent. Postmortem examination reveals cerebral edema and areas of demyelination. Survivors have brain damage necessitating psychiatric or institutional care.
2. Vaccinia necrosum (progressive vaccinia). Progressive vaccinia occurs in those with impaired cell-mediated or humoral immunity. The complication is characterized by spreading necrosis at the vaccination site and the appearance of metastatic lesions in various other parts of the body including internal organs. The disease is usually fatal.
3. Eczema vaccinatum (Fig. 34-3). Vaccination of patients with clinically apparent eczema, or even those in remission, can result in transfer of virus to other skin lesions. The condition appears to be more severe in patients accidentally infected by contact with a recently vaccinated person.

Fig. 34-3. Eczema vaccinatum. (Courtesy Department of Pathology, University of Illinois Medical Center, Chicago.)



There are very few fatalities from eczema vaccinatum.

4. Generalized vaccinia. No serious sequelae result from generalized vaccinia. It is the consequence of transitory viremia in which lesions may appear over most of the body. Underlying conditions such as skin disease, immunodeficiencies, and steroid therapy may predispose the patient to generalized vaccinia.
5. Autoinoculation. Residual vaccine picked up from the vaccination site is often the source of accidental infection. Most infections occur in the eye or eyelids. Unless the cornea is involved, autoinoculation has no serious sequelae. (See boxed essay, p. 716.)
6. Granuloma pyogenicum. Several reports from the Far East have described the development of bright red lesions at the vaccination site. The globular lesions are circumscribed and have a central exudative ulceration. There are no serious complications from this condition, which many clinicians believe to be due to hyperplasia (abnormal increase in cells) at the site of inoculation.
7. Fetal vaccinia. Fetal vaccinia is a result of maternal vaccination between the third and twenty-fourth weeks of pregnancy. Because of the high incidence of stillbirths, maternal vaccination is no longer recommended.

THE HERPESVIRUSES

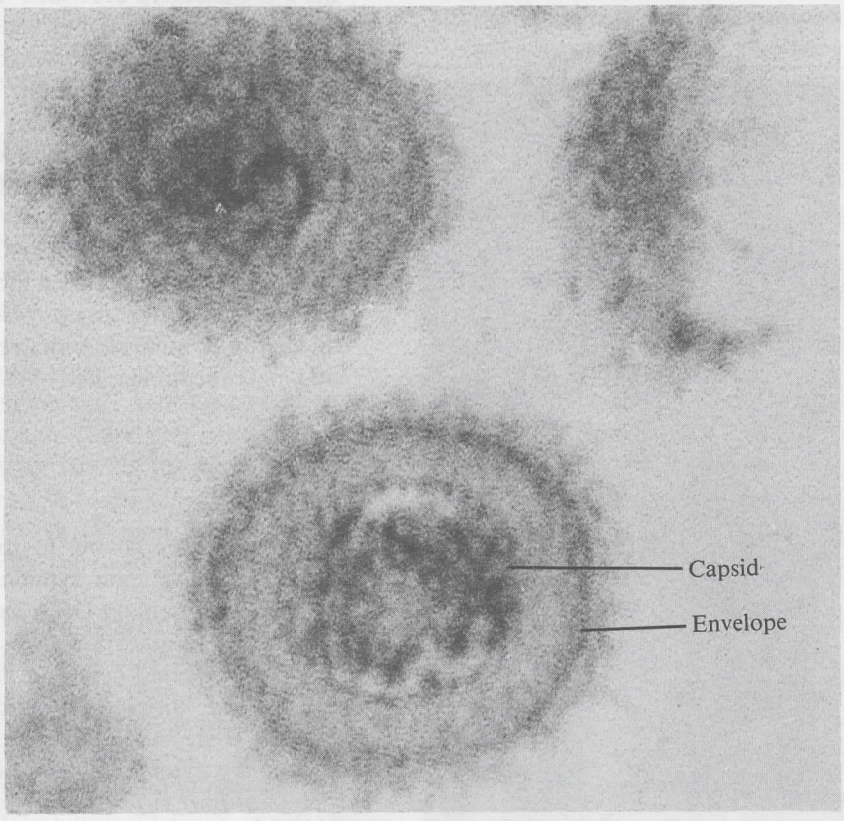
The herpesviruses belong to the family Herpesviridae. They have cubic symmetry, are enveloped (Fig. 34-4), and contain DNA as their genetic material. Viral multiplication takes place in the nucleus of the infected cell, and the envelope is acquired as the virus buds through the nuclear membrane (Fig. 34-5). The important members of this group are *cytomegalovirus, varicella-zoster virus, Epstein-Barr virus*, and *herpes simplex viruses*. All herpesviruses are characterized by their ability to remain in a latent state and are therefore associated with recurrent infections.

Cytomegalovirus

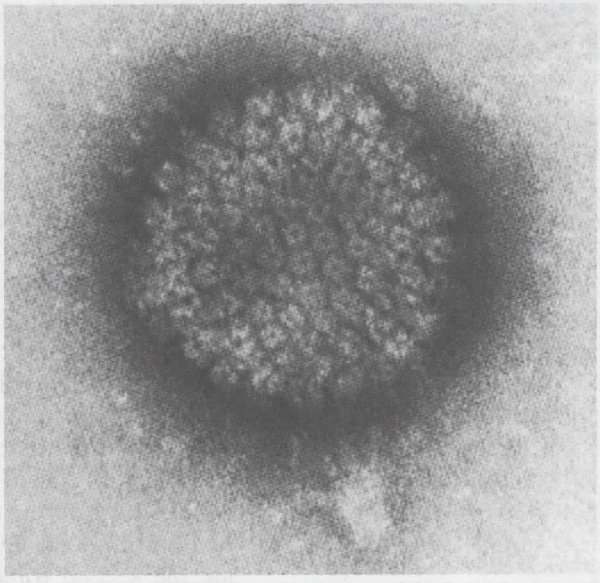
PATHOGENESIS. Most susceptible to infection by cytomegalovirus (CMV) are the fetus and the renal transplant patient.

Infection in the Newborn. Pregnant women may acquire CMV by primary infection or by reactivation of a latent infection. Primary infection in the mother results in viremia, and the virus can pass through the placenta and infect the fetus. Nearly every organ of the fetus becomes infected with virus. Unlike rubella virus infection, which has its worst effects during the first trimester, CMV infection has more deleterious effects on the fetus during the second trimester of pregnancy. It is esti-

Fig. 34-4. Electron micrographs of herpesvirus. A. Intact herpesvirus particles showing envelope surrounding capsid. (Courtesy B. Roizman.) B. Electron micrograph of the herpesvirus capsid. Note arrangement of the capsomeres. (From R. C. Williams and H. W. Fisher, *An Electron Micrograph Atlas of Viruses*, 1974. Courtesy Charles C Thomas, Publisher, Springfield, Illinois.)

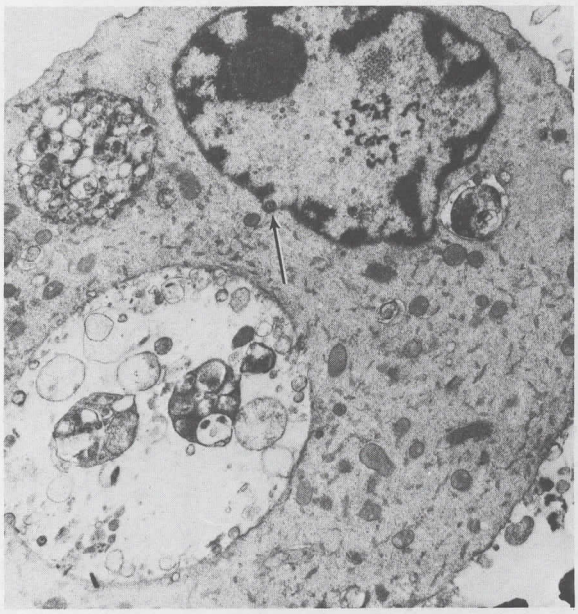


A

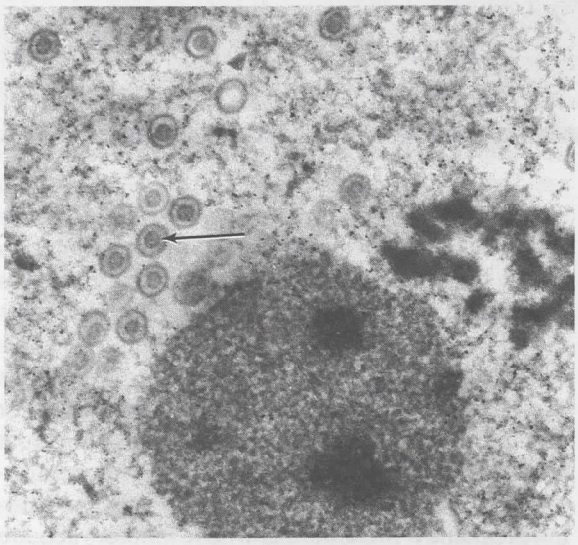


B

Fig. 34-5. Electron micrographs of herpesvirus budding through nuclear membrane of a macrophage. A. Nucleocapsid (*arrow*) of virus budding at nuclear membrane (× 19,500 before 32% reduction) B. Enlargement (× 123,000 before 35% reduction) of nuclear portion of A, demonstrating capsids (*arrow*) of virus. (From K. Sethi et al. *Arch. Virol.* 59:157, 1979.



A



B

mated that approximately one percent of all infants are born congenitally infected with CMV. From 5 to 10 percent of congenitally infected infants manifest symptoms, which may include intrauterine growth retardation, hepatosplenomegaly, jaundice, and neurological manifestations including deafness. A frequent result of symptomatic infection is permanent mental retardation. Childhood retardation from congenital CMV infections is estimated at 2.5 to 10 per 1000 live births.

The most common source of perinatal or postnatal infection is reactivation of latent infections in the mother. Intrauterine infections after reactivation are uncommon because of circulating maternal antibody. Virus may be excreted by the mother from the cervix, in breast milk, and in urine and throat secretions. The infant becomes infected during passage through the cervix or acquires the virus from the mother's mouth or breast milk. These infections are less severe than congenital infections and cause mild or marked respiratory disturbances and some liver malfunction. Approximately 2 to 3 percent of all infants are infected perinatally with CMV.

Infection in the Renal Transplant Patient. CMV infection is common after renal transplantation (more than 90 percent). The virus may be transmitted via an infected donor kidney, or infection may occur by reactivation of latent virus. Reactivation is sometimes due to immunosuppressive therapy, which is part of the transplantation procedure, or to host-vs.-graft rejection. CMV infection may result in two different syndromes:

1. Six months after transplantation a benign, self-limiting disease may appear, accompanied by fever, leukopenia, and biopsy evidence of rejection. Antibodies to CMV are present in the serum. The deteriorating allograft function improves when immunosuppressive therapy is halted.
2. The same symptoms of fever and leukopenia appear as in the first syndrome but are soon followed by prostration, severe pulmonary and hepatic dysfunction, muscle wasting, central nervous system depression, and death. Antibod-

DISSEMINATED VACCINIA INFECTION IN A COLLEGE STUDENT—TENNESSEE

On October 12, 1982, the University of Tennessee Student Health Service notified the Tennessee Department of Public Health that a 19-year-old male undergraduate had been hospitalized that day for disseminated vaccinia infection.

The student was vaccinated for the first time in his life at an Air National Guard meeting in Nashville on October 3, 1982. A primary take appeared at the vaccination site on October 5, after his return to the university in Knoxville. On October 9, multiple pustules developed on his face. On October 12, the patient's right upper arm was swollen and erythematous, with a 2- to 3-cm vaccinial lesion and exquisitely tender right axillary nodes. He had numerous confluent facial lesions compatible with vaccinia on both cheeks in areas of active acne. He also had anterior cervical and submandibular lymphadenopathy. The patient appeared acutely ill with chills and a temperature of 38.7°C (101.7°F). Vaccinia immune globulin (VIG) was obtained from the Centers for Disease Control, and 25 ml, half the indicated dose, was administered intramuscularly that evening. By morning, the patient appeared much improved; he was afebrile, and axillary tenderness was markedly decreased. No additional VIG was given. The patient continued to improve over the next 5 days and returned to class on October 18. No secondary cases were identified.

This student probably inoculated vaccinia virus from the smallpox vaccination site to the acne on his face. Normal skin is rarely infected by vaccinia virus shed from smallpox vaccination. Abnormal skin such as that affected by atopic dermatitis is more susceptible to infection by inoculation.

From *MMWR* 31:682, 1982.

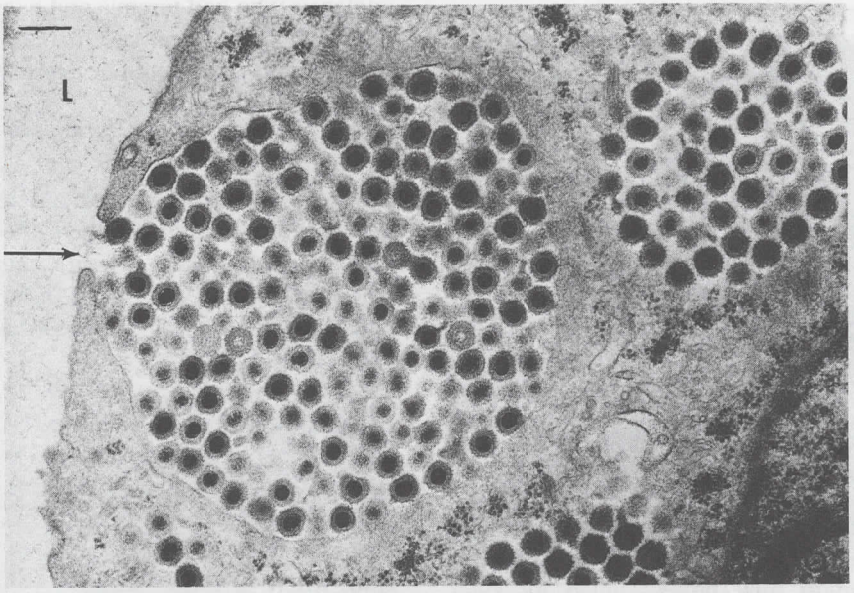
ies to CMV do not develop, and allograft biopsy shows no evidence of rejection.

Virus shedding occurs in 40 to 80 percent of transplant patients and is more common in those who have antibody to CMV before transplantation. Of those who shed virus, less than one-third have illness. The parent-to-offspring transplant pairing produces more primary infections in recipients than does cadaver-to-recipient pairing.

In patients receiving massive transfusions (for example, during heart surgery), a mononucleosis- or hepatitis-like syndrome may appear. Because CMV is found in the leukocytes it is widely believed that the CMV in the donor leukocytes is reactivated in the recipient.

EPIDEMIOLOGY. Approximately 1 percent of infants excrete CMV at birth and continue to shed it for months or even years in the urine or from the throat (Fig. 34-6). Symptomatic congenital infection appears to result from a primary CMV infection during pregnancy. Most infants whose mothers acquire CMV before pregnancy are asymptomatic at birth

Fig. 34-6. Electron micrograph of cytomegalovirus in vacuole of guinea pig salivary gland duct cell. The vacuole opens (*arrow*) directly into salivary gland duct lumen (*L*), releasing mature virions. (Courtesy C. K. Y. Fong. From F. J. Bia, et al., *Rev. Infect. Dis.* 5:177, 1983.)



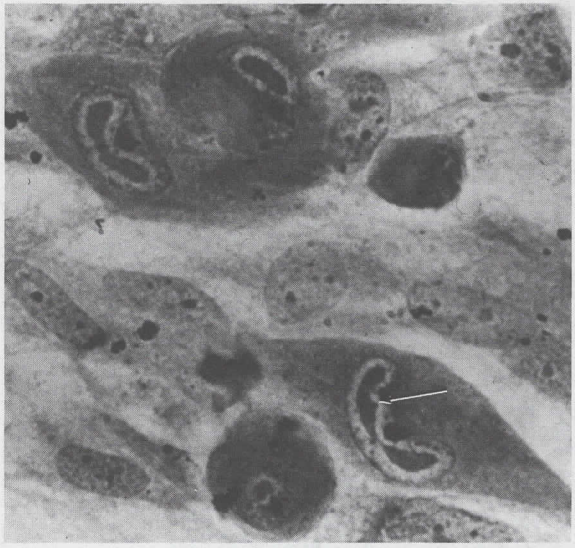
even though some show a tendency to hearing loss. Less than 50 percent of the infants whose mothers acquire primary CMV infections during pregnancy are symptomatic at birth.

The incidence of infection in those who are not congenitally infected increases with age and is correlated with socioeconomic status—the lower the socioeconomic status the greater the incidence of infection. Most children and adults who acquire infection develop a subclinical infection, and less than 2 percent of these develop CMV-associated mononucleosis. Excretion of virus by this group may persist for months or even years after symptoms have dissipated. In addition to mononucleosis, CMV infection can cause pneumonia, hepatitis, pericarditis, and encephalitis. The role of CMV in the immunosuppressed, including transplant and leukemic patients, was discussed earlier.

The mode of transmission of CMV is not completely understood. The virus is relatively unstable outside the host or body fluids. Few laboratory workers or hospital personnel who are exposed to the virus demonstrate disease. It is therefore believed that CMV is spread by close personal contact, including congenital transfer, direct contact with donor organs or blood transfusions, and contact with body fluids of someone who sheds the virus.

LABORATORY DIAGNOSIS. Detection of primary infection by CMV is difficult because most infections are asymptomatic. Pregnant women who are seronegative would have to undergo testing for CMV for up to 20 weeks, a difficult and expensive process. Diagnosis of CMV infection requires laboratory techniques: (1) assay of complement-fixing or neutralizing antibody titer (a fourfold rise indicates infection); (2) isolation of virus from bronchial washings, urine, peripheral blood, or other specimens by cultivation in human fibroblast cell cultures (a typical viral inclusion body is observed in culture—Fig. 34-7); and (3) biopsy examination of lung, liver, and intestinal tract for viral inclusion bodies. For detection of potentially lethal CMV in-

Fig. 34-7. Typical enlarged cytomegalovirus-infected human embryonic fibroblast cells in culture with intranuclear type A inclusions (*arrow*) and cytoplasmic lesion. (Courtesy H. Yamashiroya.)



fections early during their course in transplant patients, biopsy is the best method of diagnosis. Kits that detect CMV IgG are available from commercial sources.

PREVENTION AND TREATMENT. There is no specific treatment for CMV infection. Whenever possible, kidney transplant patients who are seronegative for CMV should receive kidneys from individuals who are also seronegative. The best answer to prevention is a vaccine. The vaccine might establish a latent infection, but in the immunologically mature individual this probably would not lead to any unusual incidence of reactivation. If reactivation did occur in pregnancy, the preexisting level of antibody would prevent congenital infections. A live vaccine is currently being evaluated. The Towne 125 attenuated CMV vaccine strain has been used on a few CMV-seronegative patients before renal transplantation. The vaccine stimulated CMV-specific antibodies in 14–28 days. Transplantation of a kidney from a seropositive donor 65 days later did not cause virus reactivation, and there was no evidence of viral shedding. Vaccine has also been administered to patients with CMV-associated mononucleosis who had viremia and excreted virus from multiple sites. After vaccination neither viremia nor viral excretion could be detected.

Varicella-Zoster Virus (Chickenpox and Shingles)

Chickenpox and shingles are different clinical manifestations of infection by the same virus. Chickenpox (*varicella*) is a benign childhood disease, whereas shingles (*zoster*) is a disease of later life in which reactivation of the latent varicella infection has occurred.

PATHOGENESIS. *Varicella.* Varicella infection begins in the upper respiratory tract. The incubation period is between 15 and 18 days. Replication of the virus occurs in the respiratory tract, and viremia ensues. From the blood the virus migrates to the skin, where a rash arises, followed by vesicle formation (Fig. 34-8). The lesions are confined to the thoracic, lumbar, and facial areas. The vesicles of varicella differ from those of smallpox in that all stages of vesicle development may be seen at any one time on the body of the individual infected with varicella. One week after their appearance the vesicles become crusted and fall off, with no visible scarring. The infectious period begins 1–2 days before the rash and lasts up to 1 week after lesions appear. During vesicle development, virus is transported to cranial nerves and dorsal root ganglion cells by centripetal movement along sensory nerve fibers.

Chickenpox is usually regarded as a benign, self-limiting disease. The most common cause of morbidity in normal children is bacterial superinfection. Severe infections caused by either streptococci or staphylococci may occur and may be accompanied by gangrene and deep vein thrombosis. Varicella encephalitis is an infrequent complication. Varicella is more severe in normal adults, and pneumonia has been reported in as many as 16 percent of those infected. In children and adults who

Fig. 34-8. Chickenpox lesions at various stages of development. (Courtesy Department of Pathology, University of Illinois Medical Center, Chicago.)

have immunodeficiency disease or who are being treated with immunosuppressive drugs, varicella can become disseminated and is a life-threatening infection. The incidence of disseminated disease in the immunosuppressed is as high as 30 percent, with a mortality as high as 7 percent.

Varicella has teratogenic potential (ability to cause congenital abnormalities) when contracted during the first trimester of pregnancy. If the mother is infected during later stages of pregnancy, the infant develops a rash at birth or shortly thereafter but no congenital anomalies appear. Some of the effects of early fetal infection are low birth weight, bilateral cataracts, an atrophic limb, cortical atrophy, and seizure activity.

Zoster. Zoster is attributed to the activation of latent varicella virus that is initially in the cells of the posterior root or cranial sensory nerve ganglia. Its incidence and severity increase with age, and it seldom occurs in the young. Many factors may be responsible for viral reactivation: corticosteroid therapy for nonmalignant diseases, fatigue, sunburn. Once the virus is activated, it moves down the sensory nerves until it reaches the skin, where it replicates and produces localized lesions similar to those of chickenpox. The lesions are confined to the areas affected in varicella—thoracic, lumbar, and facial areas. Before the appearance of the lesions a generalized rash may arise accompanied by fever and intense pain over the involved nerve. In approximately 3–5 weeks the lesions heal and the pain subsides.

Postherpetic neuralgia (pain after healing of vesicles) frequently occurs in adults more than 60 years of age and is believed to be due to scarring in the ganglia and afferent portions of the sensory nerve. The symptoms may last as long as 6 months. Facial nerve paralysis is a common complication of zoster. It is not permanent and is resolved within 2–3 months after infection.

Zoster may disseminate into various organs, particularly the lungs, in a few patients who have some underlying illness such as Hodgkin's disease or who are being treated with immunosuppressive drugs. Untreated disseminated zoster has a high mortality, resulting most frequently from viral pneumonia or secondary bacterial infections.

EPIDEMIOLOGY. Varicella is a very contagious disease found predominately in children, although other age groups may be affected. The disease is believed to be transmitted by the airborne route. The clinical manifestations of chickenpox, as opposed to zoster, are believed to represent the individual's first encounter with the herpes agent. The greatest incidence of the disease is during the spring months and among children 5–8 years old. More than 80 percent of the adult population give a history of having had a childhood attack of varicella.

Zoster occurs primarily in adults and is neither as seasonal nor as infectious as varicella. Zoster is generally thought to result from the reactivation of virus in those who have had varicella. The frequency and severity of the shingles increase with age, and second attacks are not uncommon. Zoster has been known to give rise to varicella, particu-

larly in nonimmune children who come in contact with zoster patients.

LABORATORY DIAGNOSIS. Varicella can ordinarily be diagnosed from the rash or history of recent exposure. The rash can be confused with the smallpox rash, however, and specific laboratory diagnosis is important. In addition, when varicella pneumonia or other complications are evident, a rapid and specific diagnosis is crucial.

A presumptive but rapid test for identification of the varicella-zoster virus involves examination of vesicular material. The degenerated cells at the base of fresh vesicles contain inclusion bodies that can be detected by Giemsa staining. This method serves to distinguish between the poxviruses and the varicella-zoster virus. Presumptive identification can also be made by electron microscopy of vesicular material. Differentiation between varicella-zoster virus and other herpesviruses such as the herpes simplex virus can be accomplished by immunofluorescent staining or gel diffusion tests. In the immunofluorescent staining method, fluorescein-conjugated immunoglobulin from hyperimmune animal serum is used.

The complement fixation test performed on sera from convalescent and acutely ill patients is employed in the serological diagnosis of varicella-zoster virus infection. The diagnosis is indicated if there is a fourfold increase in complement-fixing antibody titer of the convalescent-stage serum compared with that of the acute-stage serum. The complement-fixing titer to varicella-zoster antigen should also be compared to the titer to herpes simplex antigen to differentiate between herpes simplex and varicella-zoster infection.

IMMUNITY. Natural infection or immunization results in the development of varicella-zoster virus–specific IgG and IgM antibodies in the serum. Antibody activity is four to eight times higher after natural infection than after immunization. Antibody that is produced persists for many years, crosses the placenta, and is present before the onset of zoster. Development of serum and nasopharyngeal IgA antibodies occurs with natural infection but not after immunization. Cell-mediated immunity increases immediately after infection and persists for several years. As with other viruses cell-mediated immunity plays a more important role in recovery from infection than does humoral immunity.

PREVENTION AND TREATMENT. Treatment is not necessary in uncomplicated varicella or zoster other than relieving the itching of varicella or the pain of zoster. Preliminary clinical studies have indicated that vidarabine (adenine arabinoside or ara-A) can be used successfully in complicated cases of varicella, especially varicella pneumonia. The drug should be restricted to immunologically normal patients because it has no therapeutic effect on varicella pneumonia patients compromised by immunodeficiencies. Further clinical trials must be performed with this drug since in some patients its use has caused a decrease in the hemoglobin level.

Immune globulins have been shown to be effective in preventing or modifying varicella infection. Two immune globulins are currently available. *Zoster immune globulin* (*ZIG*) is prepared from the plasma of persons recovering from zoster. *Varicella-zoster immune globulin* (*VZIG*) is prepared from outdated blood bank plasma from normal persons with high titers of varicella-zoster antibody. Both immune globulins are in short supply and not always available. ZIG and VZIG can prevent varicella in normal children but can only ameliorate the illness of immunosuppressed children. Two groups are eligible to receive ZIG or VZIG: (1) susceptible children with high-risk conditions such as immunodeficiencies or those receiving immunosuppressive drugs who have been exposed to confirmed cases of varicella within the previous 72 hours, and (2) neonates at high risk of having congenital varicella—that is, infants born within 4 days after onset of rash in the mother.

An attenuated varicella-zoster vaccine (OKa strain) has been experimentally evaluated in Japan. It was administered to healthy children, chronically ill children (some of whom were receiving steroid therapy), chronically ill children after the appearance of chickenpox in the ward, and seronegative

household contacts within 3 days of exposure. The vaccine proved effective in all cases by preventing infection. Antibody was detectable 2 years after vaccination. Many investigators in the United States believe that further evaluation is needed before licensing the vaccine. Much of their apprehension stems from the possibility that the vaccine itself could cause zoster (and it would take decades to find out), the immunity may not be long lasting, and the result could be a more severe disease later in life. The vaccine is under investigation in the United States.

Herpes Simplex Virus

Herpes simplex virus (HSV) is the causal agent of several human infections including cold sores, fever blisters, keratitis (inflammation of the cornea), encephalitis, and a venereal infection of both men and women. The virus may also be transmitted to the fetus, resulting in a generalized or severe infection. Recurrent herpes simplex infections are common.

There are two serotypes of HSV: types 1 and 2. *Type 1* is associated primarily with lesions above the waist. *Type 2* infections are primarily below the waist and are venereal in origin.

PATHOGENESIS. HSV infection may be primary (an acute infection that runs its natural course) or recurrent. The cause of recurrent infections is believed to be reactivation of endogenous latent virus. Thus the virus may remain dormant in nervous tissue. Supporting evidence comes from studies in which type 1 virus was isolated from the trigeminal ganglion and type 2 from the sacral ganglia of humans. It has been hypothesized that after reactivation the virus in the ganglia travels along the axon of the peripheral nerve to cause a localized infection in the innervated skin. In addition to involvement of the sensory and autonomic nervous systems, HSV is believed to be involved in motor nerves and the central nervous system motor centers. Recent studies in animal models indicate that HSV can penetrate the neuromuscular junction, travel in a pure motor nerve, and produce a focal encephalitis.

Type 1 Infections

1. *Acute herpetic gingivostomatitis* is characterized by ulcerative lesions on the mucous membranes of the oral cavity. The disease is self-limiting and is resolved in 2–3 weeks (Fig. 34-9). This is a common clinical entity.
2. *Eczema herpeticum* results from accidental inoculation of the virus into the skin lesions associated with eczema. Recurrent attacks are less severe than the initial attack.
3. *Herpetic keratoconjunctivitis* is believed to be the leading cause of loss of vision caused by external eye disease in the United States. Recurrent infections are common and often lead to opacity of the cornea and eventually to impaired vision (Fig. 34-10).
4. *Herpes labialis* is the most prevalent form of herpes type 1 infection. Infections involve the mucocutaneous junction of the lips (fever blisters and cold sores) (Fig. 34-11). Recurrent infections are common and are probably due to activation of latent virus in the sensory cells of the trigeminal nerve ganglion. Many investigators feel that conditions of stress, sunlight, hormones, or menstruation may activate the latent virus. Dental extraction appears also to trigger reactivation of latent HSV in occasional susceptible patients by axonal injury from the local anesthetic injection or by irritation of the nerve terminals from surgical procedures.
5. *Herpetic encephalitis* is the most severe type 1 infection. The disease may be the result of disseminated herpes simplex in the newborn or may be limited to the brain, especially in adults. The virus causes necrosis of both lobes of the brain and inflammation of the meninges. Most patients die within 3 weeks of onset of symptoms.

Type 2 Infections. Type 2 infections are associated with lesions below the waist. There are two manifestations of type 2 infections: herpes genitalis and neonatal herpes.

1. *Herpes genitalis* infection in women generally

Fig. 34-9. Gingivostomatitis caused by herpesvirus infection. (From J. C. Overall, Jr. In G. J. Galasso, T. C. Merigan, and R. C. Buchanan [Eds.], *Antiviral Agents and Viral Diseases in Man*. New York: Raven, 1979.)

Fig. 34-10. Herpetic keratoconjunctivitis. Dendritic ulcer stained with fluorescein in cornea of patient with recurrent disease. (Courtesy J. Sugar.)

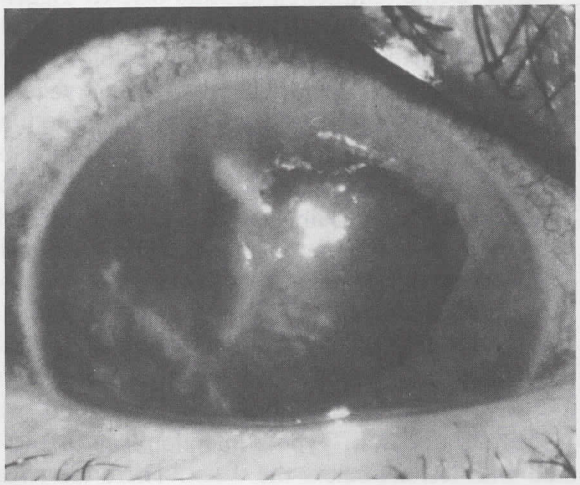


Fig. 34-11. Cutaneous lesions on lips and skin around the mouth of individual with recurrent herpes labialis. (Courtesy L. J. LeBeau.)

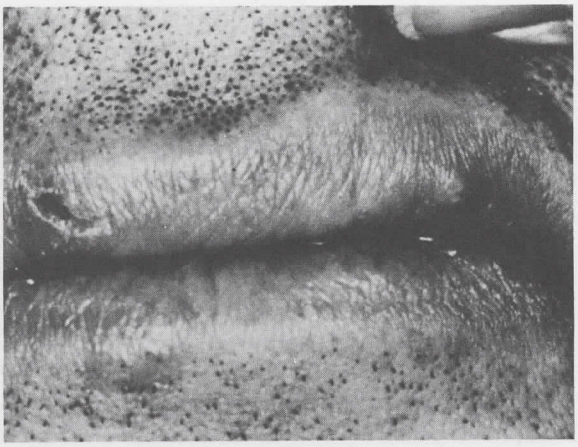


Fig. 34-12. Genital lesions caused by herpes simplex virus infection. (Courtesy L. Solomon, Department of Dermatology, University of Illinois Medical Center, Chicago.)

results in symptoms that do not require medical attention. (In some cases, however, it can be a serious disease causing severe tissue inflammation and pain.) Lesions appear on the vagina, cervix, or vulva and are only mildly discomforting. In men the lesions, which appear primarily on the penis, are painful and associated with a watery discharge (Fig. 34-12).

2. *Neonatal herpes*—herpes simplex infection in the newborn—can cause illness of variable severity. The disease is transmitted from mother to fetus during passage through the birth canal. The symptoms, which appear 3–14 days after birth, are loss of weight, vomiting, and diarrhea. In disseminated infections death is frequently due to excessive brain damage.

Other Manifestations. Lesions on the fingers or hands of physicians, dentists, and medical personnel have been called *herpetic whitlows*. They were originally thought to be caused by type 1 virus acquired through contact with oral lesions of patients. Now it has been discovered that they also occur on the foot and that both types 1 and 2 may be involved. Since type 2 is associated with genital herpes, it is suspected that finger contact with genital lesions is one means of transmission to any part of the body, including the eyes. A burning or stinging sensation is felt in the lesions. A vesicle is formed, surrounded by a rim of edema and erythema. The vesicles remain unchanged for up to 3 weeks, at which time they begin to heal.

EPIDEMIOLOGY. Herpes simplex infections affect populations throughout the world. It has been difficult to distinguish the true incidence of a primary infection from recurrent disease. Although HSV 1 is associated primarily with oral disease and HSV 2 with genital disease either strain may be involved with genital or oral disease. It is estimated that 10 percent of genital disease may be caused by type 1 strains. Recurrences of genital herpes are less likely with type 1 strains than type 2 strains. As yet no reasonable explanation for this characteristic has been forthcoming.

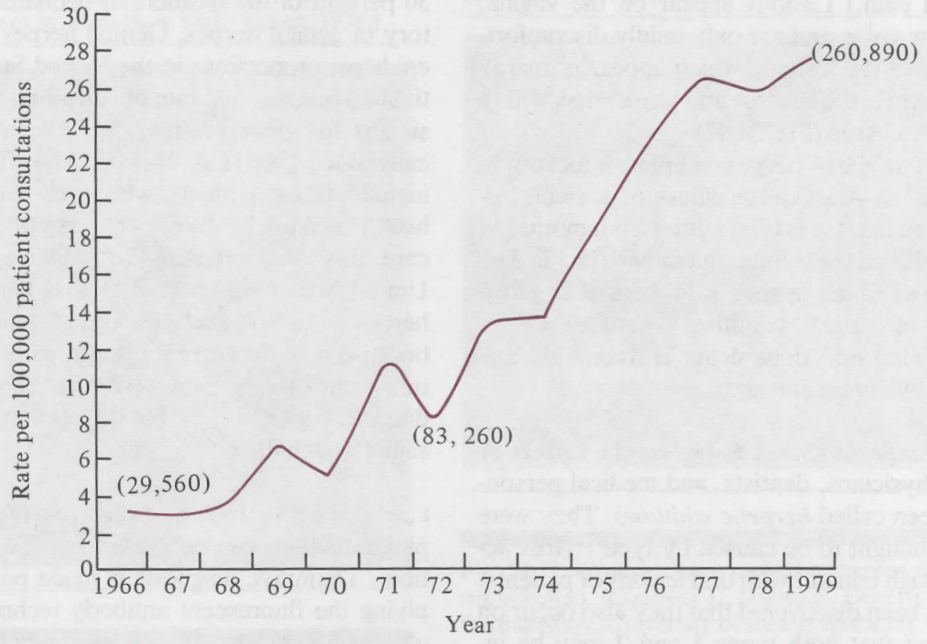
The prevalence of genital HSV infection in women during pregnancy and at term, when transmission to the neonate occurs, has not been clearly defined. Most studies indicate that approximately 30 percent of the mothers of neonates have a history of genital herpes. Genital herpes has reached epidemic proportions in the United States. The estimated number and rate of consultations with physicians for genital herpes has increased dramatically since 1966 (Fig. 34-13). These figures do not include those patients who seek care in public health facilities or those who do not seek medical care. It is estimated that 1 to 2 million people in the United States suffer from genital herpes. Genital herpes is an especially important venereal disease because it is a recurrent disease without a specific treatment or cure and newborns can acquire the disease, which may be life-threatening, during passage through the birth canal.

LABORATORY DIAGNOSIS. Diagnosis of herpes simplex infections can be made from clinical observations. Definitive diagnosis is made possible by applying the fluorescent antibody technique to cells obtained from herpetic lesions. The enzyme-linked immunosorbent assay (ELISA), however, appears to be more specific than immunofluorescence and is faster and more economical. The virus is easily isolated in cell culture, and identification can be established by reaction with specific antisera. If serum samples are available from the patient, serological diagnosis can be made employing the complement fixation technique.

Rapid diagnosis to stem herpetic encephalitis is very important. Passive hemagglutination tests can be applied to cerebrospinal fluid. Passive hemagglutinating antibodies are found in the cerebrospinal fluid during infection and are capable of agglutinating sheep red blood cells that have herpesvirus fixed to them. The test can be read in just a few hours.

PREVENTION AND TREATMENT. Effective treatment of herpes simplex infection should be concerned not only with reducing the severity of primary infection but with reducing the severity of recurrent disease, reducing transmission to contacts, and reducing the

Fig. 34-13. Estimated rate of patient consultations with private physicians for genital herpes infection, United States, 1966–1979. (Numbers in parenthesis are exact numbers.) (From *MMWR* 31.137, 1982.)



frequency of recurrences. A wide variety of drugs have been investigated, but none has proved totally effective.

Vidarabine (ara-A) is available in ophthalmic ointment and drop form and is effective in the treatment of 80 percent of the cases of corneal herpes simplex disease. Ara-A can be administered intravenously for use in treating herpes simplex encephalitis. It reduces mortality and morbidity, especially for younger patients. The drug is also being used to treat neonatal herpes simplex infections and severe mucocutaneous infections in immunocompromised patients. Idoxuridine (IDU) is an alternative to ara-A for topical treatment of keratoconjunctivitis. Trifluridine is a new drug that in clinical trials is curative in over 90 percent of the cases of keratoconjunctivitis.

Acyclovir is available as an intravenous or oral topical formulation. The drug, when administered intravenously, reduces viral shedding and hastens healing in mucocutaneous infections in immunocompromised patients and in normal individuals with primary episode genital infections. The drug is relatively ineffective in reducing the time of recurrences of infection. Topical acyclovir for first episode genital infections decreases the duration of viral shedding and reduces the time required for healing. The drug does not influence subsequent recurrence rates. An oral form of acyclovir is now available, and it has been shown to reduce the severity of disease and to reduce virus shedding.

A herpes simplex vaccine has been developed in West Germany, but the results of its trials are as yet unconvincing and it has not been approved for use in the United States. A herpes simplex subunit vaccine has been developed in the United States and is currently being evaluated.

A variety of vaccines (smallpox, yellow fever, polio, and influenza) have been used in uncontrolled studies to stimulate the immune system to

combat HSV infection. None has been shown to be effective. Interferon has also been used to stimulate immunity to HSV infection, but clinical trials have not been promising. These results are not unexpected since titers of antibody and effective cell-mediated immunity responses are normally at high levels in those with recurrent disease.

Epstein-Barr Virus and Infectious Mononucleosis

Seroepidemiologic surveys indicate that the Epstein-Barr virus (EBV) is widely disseminated throughout the world. Infection in the very young goes undetected or is very mild. In contrast, infection of adolescents and young adults results in a clinically apparent response called *infectious mononucleosis*. A study of colleges has revealed that 50 percent of the students with primary EBV infection develop infectious mononucleosis. After primary infection the virus persists in the host indefinitely—latent EBV infection is prevalent in all populations, and virus may remain latent in some B lymphocytes. Virus may be shed in oropharyngeal secretions after primary infection, and infection may recur with immunosuppression. Lymphomas similar to Burkitt's lymphoma may develop in some immunosuppressed children. Infected B lymphocytes when cultured are capable of sustained growth; however, uninfected cells have a limited life span. Most adults have B cells infected with EBV, but the cells do not become malignant.

Infectious mononucleosis symptoms may appear 5–50 days after infection. Symptoms initially include headache, fatigue, fever, and chills. Mild cases are characterized by a febrile period with cervical lymph node enlargement. In more severe cases the fever lasts 8–10 days with cervical lymph node enlargement and hepatosplenomegaly. During the course of disease the white blood cell count may be as high as 75,000/mm^3.

EBV nuclear antigen and genome are present in almost all cells affected by the African strain of Burkitt's lymphoma (see Chapter 33, Epstein-Barr Virus). Since infection does not always result in malignancy of B lymphocytes, what is the trigger for Burkitt's lymphoma? Some investigators believe that a cocarcinogenic factor promotes malignancy. Others believe that the greater prevalence of lymphoma in equatorial Africa is due to the presence of an EBV isolate that differs from the American strain.

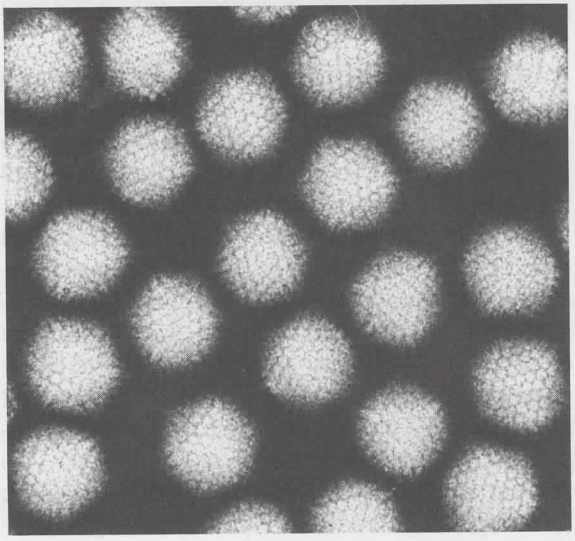
EBV has been associated with a second human malignancy, nasopharyngeal carcinoma. This disease is infrequently found worldwide and has a high incidence only in southern China. Environmental factors may play some role in this endemic disease.

Infectious mononucleosis may be diagnosed based on clinical symptoms plus the findings of elevated lymphocyte levels and the presence of atypical lymphocytes. Orally excreted virus can be detected by its ability to alter the morphologic properties of cord blood lymphocytes. Fluorescent antibody is the method of choice for serological diagnosis. *Heterophile antibody* is found in up to 6 percent of patients with infectious mononucleosis. (Heterophile antibodies are capable of agglutinating sheep red blood cells or horse red blood cells. These antibodies are nonspecific and can be produced in other conditions.) A rapid differential slide test (spot test) is available for detecting heterophile antibodies.

THE ADENOVIRUSES

The adenoviruses belong to the family Adenoviridae. They can be found in many animal species including humans. There are approximately 39 immunologically distinct adenoviruses of human origin. The virion is made up of 240 nonvertex hollow capsomeres called *hexons* (surrounded by six capsomeres) in an icosahedral pattern of cubic symmetry (Fig. 34-14). In addition there are 12 vertex capsomeres called *pentons* (surrounded by five capsomeres). Each penton has a fiber attached to it. Some adenovirus types have a knob attached to the penton fiber, and the complete virion gives the appearance of a laboratory-constructed earth satellite. The penton fibers and the terminal knobs can be seen in electron micrographs (see Fig. 33-23). The penton fibers are antigenic and probably serve as organs of attachment. They are responsible for

Fig. 34-14. Electron micrograph of adenovirus (×180,000). (Courtesy R. C. Williams.)



the hemagglutinating activities of the virus (only type 18 fails to hemagglutinate).

Pathogenesis

Adenoviruses have an affinity for mucocutaneous surfaces and are usually associated with respiratory illness, particularly in infants. In some instances an association with one clinical syndrome has been demonstrated, but for the most part adenoviruses are associated with a variety of clinical syndromes. Except for pneumonia, most adenovirus infections are not fatal. Occasionally adenoviruses are the cause of meningoencephalitis, acute hemorrhagic cystitis, and neonatal sepsis. Table 34-1 lists the major clinical syndromes in which adenoviruses have been implicated.

The adenoviruses were the first human viruses demonstrated to be oncogenic, but the oncogenicity has been observed only in laboratory rodents. To date there is no proof that the adenoviruses are oncogenic for humans.

Epidemiology

Adenovirus infections are associated with respiratory and ocular disease and occasionally gastrointestinal conditions. Serological surveys indicate that every child in the United States will have had at least one adenovirus infection by the age of 5 years. Respiratory disease is seldom seen in adults except for military recruits. Adenoviruses account for nearly 2 percent of all acute respiratory disease in nonhospitalized children and for 5 to 25 percent in hospitalized children. Of the 39 serological types of adenoviruses only 10 to 12 have been routinely associated with disease. Children under 6 years of age are most susceptible to infection by adenoviruses. The clinical conditions associated with infection in children include pharyngitis, bronchitis, croup, and pneumonia. Types 3, 7, and 21 have been associated with lower respiratory tract disease and "swimming pool" conjunctivitis in children under 6 years of age. Types 4 and 7 are the major cause of epidemic respiratory illness in military recruits. Although type 7 usually produces mild illness it has been associated with respiratory disease of high mortality, particularly in hospitalized children with underlying heart or respiratory illness. Types 8 and 19 are associated with keratoconjunctivitis, a very severe eye disease. Keratoconjunctivitis outbreaks occur frequently in industrial settings and in the offices of ophthalmologists (because of contaminated ophthalmic equipment or solutions).

Laboratory Diagnosis

Swabs of clinical specimens are inoculated into primary human embryonic kidney cell cultures and incubated. Cytopathic effects are analyzed after approximately 14 days. For rapid identification of an isolate, fluorescent antibody staining is used. Neutralization with type-specific antiserum is the best method for typing the adenovirus isolate. Typing is an important aspect of identification because a particular set of serotypes is associated with a specific disease.

Prevention and Treatment

The frequent outbreaks of respiratory disease caused by type 7 adenovirus in military camps led to the development of a vaccine. Live vaccines of types 4 and 7 have been developed for oral admin-

Table 34-1. Adenovirus-associated Syndromes and Viral Type Most Frequently Recovered

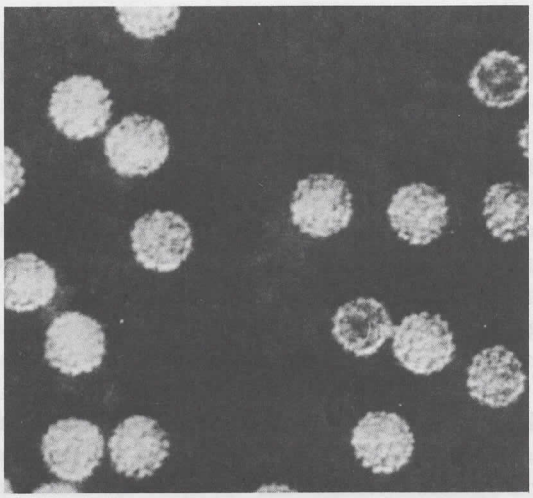
Syndrome	Adenovirus types	Symptoms and signs
Acute respiratory disease	Types 4 and 7 in military camps; types 3, 14, and 21 in civilian populations	Sore throat, cervical lymphadenopathy, fever
Pharyngoconjunctival fever	Usually types 3 and 7; types 1, 4, and 14 less frequently; worldwide summer epidemics among infants; acute follicular conjunctivitis more frequently in adults	Pharyngitis, conjunctivitis, sore throat, fever, and cervical lymphadenopathy
Pharyngitis	Types 3, 4, 7, 14, and 21	Pharyngitis and intestinal pain often observed in infants
Pneumonia	Types 3 and 7; mortality highest in this syndrome, which occurs primarily in infants	—
Epidemic keratoconjunctivitis	Types 8 and 19 most frequent cause of this syndrome in the United States	A localized infection in which subepithelial opacities occur in the cornea and may remain for up to 2 years.

istration. The virus is encased in a capsule, which when released in the intestine causes an asymptomatic response. The virus is nontransmissible. No chemotherapeutic agent has proved effective in treating any adenovirus-associated condition, including conjunctivitis.

THE PAPILLOMAVIRUSES

The papillomaviruses (Fig. 34-15) are structurally similar to the polyomaviruses, such as SV40 and the BK and JC viruses, discussed in Chapter 33, but the former cannot replicate in cultured cells. The papillomaviruses are the cause of warts. The virus infects cells and stimulates them to divide. Papillomas begin as a proliferation of connective tissue followed by a proliferation of the epidermal cells. It may take 1–6 months before the wart becomes evident. Warts are divided into four types: (1) *verruca vulgaris* (common wart), (2) *verruca plana* (flat wart), (3) *verruca plantaris* (plantar wart), and (4) *condyloma acuminata* (venereal warts). Each type is distinct and should be distinguished because therapy for each differs. Most warts regress, but if they persist they can be treated

Fig. 34-15. Electron micrograph of human papillomavirus. (From B. Janis. In P. D. Hoeprich [Ed.], *Infectious Diseases* [2nd ed.]. New York: Harper & Row, 1977.)



by electrosurgery or cryosurgery or by topical application of drugs. Podophyllum is used topically for all warts except venereal warts.

Venereal warts are soft, pink cauliflower-like growths that appear singly or in clusters on the external genitalia and rectum. The disease is one of the most common sexually transmitted diseases in the United States. There is a direct association between venereal warts and other sexually transmitted diseases. Nearly 30 percent of women with venereal warts also have gonorrhoea. Most homosexual men with perianal warts also have syphilis or gonorrhoea. Spontaneous regression of venereal warts occurs, but when they do not regress serious consequences may take place. Large growths may result in transmission to neonates, and such transmission is thought to give rise to childhood laryngeal papillomatosis. There is also some evidence that patients with a history of anogenital warts are predisposed to cervical and perianal neoplasias. Electrocauterization and cryotherapy appear to be the best methods of treatment. Recurrent warts may be removed by surgery. Intraurethral warts are best treated with the topical application of idoxuridine.

THE PARVOVIRUSES

Parvoviruses belong to the family Parvoviridae. They are nonenveloped single-stranded DNA viruses with icosahedral symmetry. The capsids have a diameter of 20 to 25 nm. Included in this group of viruses pathogenic for humans are the Norwalk agent and parvovirus B19.

Norwalk Agent

The Norwalk agent (identified during an epidemic in Norwalk, Ohio) is associated with *epidemic acute gastroenteritis*. This virus produces a disease similar to those produced by the rotaviruses, RNA viruses belonging to the family Reoviridae (see Rotaviruses later in this chapter). The virus cannot be cultivated in vitro but has been identified by immunoelectron microscopy. (Immunoelectron microscopy is a technique in which antibody specific for the virus is added to a specimen containing virus. The specific immune aggregates formed are more easy to visualize than individual virions, especially if the latter are very small.) The Norwalk agent has been associated with disease primarily in school-age children and adults. The disease is characterized by vomiting, nausea, diarrhea, and abdominal pain. Most epidemics or sporadic cases occur during the winter months, September to March.

Parvovirus B19

Parvovirus B19 was first discovered in human blood in 1975. Surveys showed that human populations were widely exposed to the virus, but no disease association could be demonstrated until 1981. Individuals with some type of anemia such as sickle cell and thalassemia appear to be particularly susceptible to infection by this virus. The virus infects rapidly dividing bone marrow cells (cell division by bone marrow cells is greatly accelerated during anemia) and thus exacerbates the anemic condition. Infection by parvovirus B19 does not appear to cause serious disease. Infection is short (7–10 days), and an active immune response terminates the illness. The severe anemia associated with disease can be treated with transfusions until the crisis is over.

RNA VIRUSES AND ASSOCIATED DISEASES

MEASLES

Measles as a clinical entity has been recognized for several hundred years. The measles virus was first cultivated in human and monkey kidney cell cultures in 1954. In 1963 an attenuated vaccine was made available to the public, and since that time the number of measles cases in the United States has dropped precipitously. In less well-developed countries, however, the morbidity and mortality from measles infection remain at high levels.

The measles virus is an enveloped, spherical

RNA virus measuring approximately 150 nm in diameter It belongs to the Paramyxoviridae family. The envelope contains hemagglutinin peplomers but does not carry neuraminidase, as many other paramyxoviruses do (see Fig. 33-17). The virus is very labile at 37°C but is stable when stored at subzero temperatures.

Pathogenesis

Measles infection is initiated in the upper respiratory tract. The incubation period lasts 7–10 days, during which time fever, conjunctivitis, sore throat, photophobia, and headache appear In the incubation period the virus multiplies in the respiratory mucosa and is then disseminated to the entire body via the bloodstream. A rash first appears on the face and then spreads to the extremities. The rash is made up of reddish elevated macules that tend to coalesce and lasts for only 3 or 4 days. Throughout the incubation period and up to 2 days after the appearance of the rash the patient is highly contagious. Virus can be shed from the conjunctiva or the respiratory mucosa.

A day or so before the rash is visible, small red macules with a bluish-white center appear on the inside of the cheek in more than 95 percent of persons infected. These lesions are called *Koplik's spots* and are important in measles identification.

Measles is a self-limiting disease; however, secondary infections of the upper respiratory tract such as pneumonia do occur, particularly in patients debilitated by other illnesses. Encephalitis occurs in 1 of every 1000 cases. A very small percentage of patients contract a demyelinating encephalitis, which is fatal to one-half of those affected. The remainder of those with encephalitis suffer varying degrees of central nervous system (CNS) injury that may result in loss of mental and motor functions. A rare sequela of measles infection is *subacute sclerosing panencephalitis* (*SSPE*) (see Slow Virus Disease later in this chapter). It is characterized by changes in personality, motor loss, speech difficulty, and mental retardation. Death is the invariable outcome of SSPE.

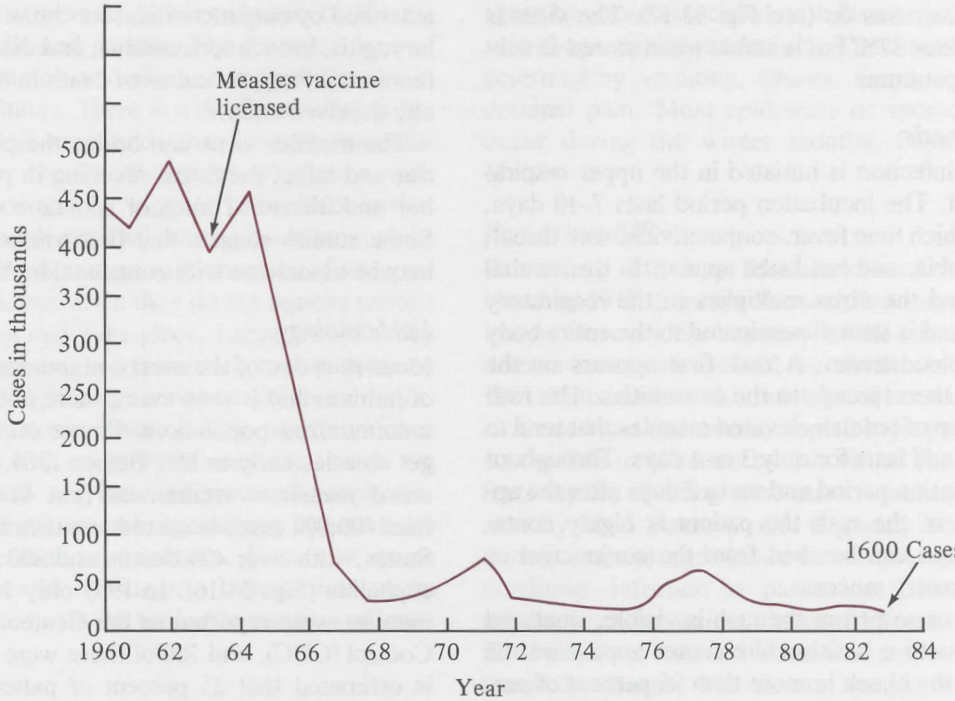
Severe forms of measles are common in areas of the world in which the children suffer from some degree of protein deficiency. Protein deficiency is a cause of suppression of the immune system and can lead to the spread of various pathogenic bacteria after measles infections. Severe measles is characterized by conjunctivitis, sore throat (stomatitis), laryngitis, bronchopneumonia, and diarrhea. Pneumonia is a frequent cause of death in the nutritionally deprived child.

The measles virus can bridge the placental barrier and infect the fetus, resulting in premature labor and increased rates of spontaneous abortion. Some studies suggest that first trimester infection may be associated with congenital malformation.

Epidemiology

Measles is one of the most communicable diseases of humans and is seen everywhere in the world. In unimmunized populations almost every child will get measles early in life. Before 1963, when attenuated measles vaccine was first licensed, more than 400,000 cases occurred annually in the United States, with over 400 deaths and 300 cases of encephalitis (Fig. 34-16). In 1983 only 1600 cases of measles were reported to the Centers for Disease Control (CDC), and 300 of these were imported. It is estimated that 25 percent of patients with encephalitis caused by measles will be left with residual brain damage manifested by conditions such as mental retardation, seizure disorders, and nerve deafness. Measles is primarily a disease of childhood, but unimmunized adults are just as susceptible as children (see boxed essay, p. 732). Epidemic cycles appear every 2–3 years and are related to climate, the season, and the number of unimmunized persons in the community. Since the widespread administration of vaccine in the United States these 2- to 3-year peaks of incidence no longer occur in this country. The administration of vaccine has also resulted in a reduction of the incidence of disease in school-age children. The highest incidence now observed in the United States is in children under 5 years of age. In unimmunized populations most infections occur in children between 6 months and 2 years of age. Spread of virus is more rapid when younger children are infected because of greater contact with other family members. In addition

Fig. 34-16. Number of reported cases of measles in the United States, 1960–1983. (From Centers for Disease Control.)



young children, especially those who are malnourished, tend to shed virus for longer periods of time than young children who are not nutritionally compromised.

Laboratory Diagnosis

Measles is diagnosed on the basis of certain clinical features—for example, Koplik's spots and rash. In many cases Koplik's spots are not detected and the measles rash may be misdiagnosed. During measles infection the virus causes the formation of multinucleate giant cells 2–6 days after the disappearance of the rash. Indirect fluorescent antibody technique can detect these giant cells in nasopharyngeal cells aspirated from the patient. The demonstration of the multinucleate giant cells is a positive test for measles. Complement-fixing, neutralizing, and hemagglutination-inhibiting antibodies develop in the serum at the onset of the rash. The hemagglutination inhibition test is one of the most useful and rapid tests for determining a patient's immunity status. The ELISA, however, is a more sensitive test.

Prevention and Treatment

The best method for control of measles is vaccination. From 1963 to 1967 an inactivated vaccine was used, and many vaccinations did not take because of nonimmunogenicity of the vaccine or because the vaccine was administered at too early an age, that is, less than 12 months. Probably as a result of this failure, cases of measles among today's 15- to 19-year-olds have increased. An attenuated vaccine, prepared from chick embryo cell culture, has been available since 1968. The vaccine currently used is called *further attenuated Edmonston B vac-*

cine. The vaccine is available in monovalent form and in combinations: measles-rubella (MR) and measles-mumps-rubella (MMR). All measles vaccines are recommended for use at approximately 15 months of age; the MMR is recommended for use in infant-child vaccination programs. Vaccine is also suggested for those who were inoculated with the inactivated vaccine in the 1963–1967 period. During measles outbreaks vaccination is also recommended for infants at 6 months of age, but they should be revaccinated at 15 months to ensure adequate immunological response. The administration of attenuated vaccine to persons who had previously received inactivated vaccine does not always provide protection, and upon exposure to natural measles virus they develop atypical measles. Fever, lymphadenopathy, and local edema have been observed. It is believed that the immune response from the inactivated vaccine interferes with the immunological response from attenuated vaccine. Vaccine should not be administered to pregnant women or to anyone with a febrile illness. A program to eliminate measles as an indigenous disease was begun in 1978 in the United States. The efforts have been successful primarily because of enforcement of school immunization requirements at all grade levels including day-care centers, where outbreaks occasionally occur. Programs are now being developed to vaccinate all children at 15 months of age.

Immune serum globulin (ISG) provides short-term protection if given less than 6 days before exposure to the virus. ISG is especially valuable for susceptible household contacts of measles patients, especially those less than 1 year old. Vaccine should not be given at the time of ISG administration; at least 3 months should elapse before vaccine is administered. ISG does not prevent infections from occurring at a later date.

MUMPS (EPIDEMIC PAROTITIS)

The mumps virus belongs to the Paramyxoviridae family. The mumps virus is a spherical, enveloped RNA virus measuring approximately 175 nm in diameter. The envelope carries glycoprotein peplomers (hemagglutinins) as well as the enzyme neuraminidase. Neuraminidase can destroy cell receptor sites and thus may interfere with the hemagglutinin reaction. There are two complement-fixing antigens associated with the mumps virus: the soluble or S antigen and the V antigen. The *S antigen* is associated with the ribonucleoprotein core of the virus; the *V antigen*, with the viral envelope.

Pathogenesis

The incubation period of mumps is 16–21 days. During this period the virus multiplies in the upper respiratory tract. Later the virus enters the blood and infects other organs and tissues including the CNS. The salivary (parotid) glands are invariably infected, with swelling and sometimes intense pain. (In the view of some researchers, the virus in the oral cavity goes to Stensen's duct and then the parotid gland, where it multiplies and finally causes a general viremia.) Unilateral infection of the testes occurs in approximately 25 percent of men infected. Infections are rare in infants up to 6 months old because of the protective influence of maternal antibody acquired in utero. Early infections of the fetus may cause death or premature onset of labor, but not congenital malformations.

Mumps is a benign disease although complications occasionally arise. These include (1) pancreatitis, which may or may not induce diabetes; (2) thrombocytopenia, a disease characterized by bleeding caused by a decrease in the number of blood platelets; (3) aseptic meningitis*, which may occur in up to 10 percent of those infected; (4) meningoencephalitis, a complication of the CNS that may afflict 0.5 to 10 percent of those infected without causing permanent damage; and (5) orchitis (infection of the testes), which may accompany 20 percent of the clinical cases of mumps in postpubertal males (unilateral infection does not usually result in sterility, but bilateral involvement poses a

**Aseptic meningitis* refers to viral meningitis, in which spinal fluid is clear. In bacterial meningitis spinal fluid is often purulent (cloudy owing to large numbers of bacteria and leukocytes).

MEASLES IN UNIVERSITIES—INDIANA, 1983

During the first 2 months of 1983, 85 clinical measles cases were reported among university students in Indiana. Four had been serologically confirmed as of February 25. The cases occurred on two of the largest campuses in the state.

At Indiana University (enrollment 32,000), 67 cases have been reported, with rash onset ranging from February 11 through February 28. Two students required stays at the Student Health Center for observation. A voluntary immunization program was initiated on February 15, and over 9000 students had been vaccinated by February 27. At Purdue University (enrollment 32,000) 18 cases have been reported, with rash onset from January 19 to February 18. Two students required hospitalization, and two other students' illnesses were consistent with atypical measles syndrome. A voluntary immunization program was initiated on February 3, and over 9000 students had been vaccinated by February 27.

As a result of these measles outbreaks the state board of health, in cooperation with local health departments and university officials, has begun a state-wide immunization campaign to increase the level of measles immunity among the approximately 200,000 college and university students. Twenty-one of the 65 independent and public colleges and universities, which contain more than 90 percent of Indiana's college student population, currently have active vaccination programs.

Measles outbreaks have been and continue to be reported from places where young adults are concentrated, such as colleges and universities. These outbreaks indicate that sufficient numbers of susceptibles may exist in college and university populations to sustain transmission when measles is introduced.

While past serological studies have indicated that overall susceptibility to measles is low, there can be considerable variation within college population subgroups. In 1977, as many as 20 percent of University of California, Los Angeles, students in some dormitories lacked detectable measles antibody. The high susceptibility rate in some college and university subpopulations is probably the result of four interactive factors: (1) Many children growing up in the mid-1960s may have missed measles vaccination in the first years following licensure; (2) many students may not have been immunized under comprehensive school laws now in effect in many states involving students in kindergarten through twelfth grade; (3) many colleges and universities lack immunization requirements; (4) many students may have escaped natural measles infection because of decreasing transmission.

Preventing measles in college-age populations is particularly important because the disease can be more serious in adults than in grade-school-age children. Persons 20 years of age and older have had the highest death-to-case ratio in recent years.

Identifying persons susceptible to measles during a measles outbreak is not only costly and a source of disruption to college routine but is also a major obstacle in control of the outbreak. In view of the importance of preventing measles in college students, the Immunization Practices Advisory Committee has recommended that officials strongly consider immunization requirements for college entry. Even before vaccination requirements are instituted, university officials should aggressively urge that all college students be protected against measles.

From *MMWR* 32:113, 1983.

risk). In females the ovaries may be infected but there is no threat of sterility.

Epidemiology

Mumps is a disease endemic throughout the world. Humans are the only known host of the mumps virus, which is transmitted by respiratory droplets or fomites contaminated with saliva. The peak incidence of disease is between January and May. Mumps is contagious primarily because as many as 50 percent of persons infected may be asymptomatic and, in those who develop symptoms, virus is shed from saliva and urine several days before and after symptoms appear. In 1967, the year the vaccine was licensed, 185,691 cases were reported in the United States, while in 1983 only 3227 cases were reported. This represents a 98 percent decrease (Fig. 34-17). Most cases occur in children before the age of 5 and up to 9 years of age; however, since 1978 the risk of infection for 10- to 14-year-olds has surpassed that for children under 5 years of age. The reason for this trend, which is also characteristic of measles and rubella, is that some individuals do not become vaccinated or have disease at a younger age.

Laboratory Diagnosis

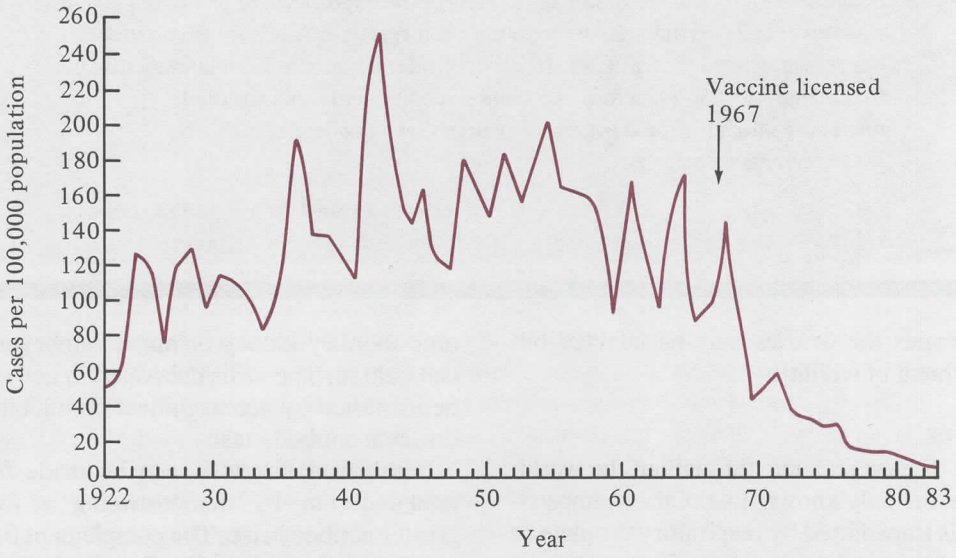
Virus can be isolated from urine, saliva, or CNS specimens within 4–5 days after onset of illness. An immunofluorescence test can be used for early diagnosis. It has been reported that virus can be isolated in 80 percent of the cases up to 15 days after onset of the disease. The specimens are inoculated onto monkey kidney or human embryonic kidney cell culture. The virus cultivated in cell culture can be identified by hemagglutination inhibition or fluorescent antibody tests.

Serological diagnosis can be made from a patient's serum by demonstrating a fourfold or greater antibody rise. The complement fixation test can detect S and V antibodies. Both S and V antigens are available commercially. V antibodies persist in the serum for years after infection and are a good indicator of previous infection. The ELISA test has a high degree of sensitivity and is the current serological test.

Prevention and Treatment

Vaccines appear to produce an immunity equivalent to that obtained in natural infection. An attenuated mumps vaccine prepared in chick embryos is available for children 1 year old or older. The vaccine is often combined with other vaccines, that is, MMR and rubella-mumps. If used with measles vaccine it should be administered at 15 months of age to ensure maximum seroconversion (antibody production) for measles (maternal antibody can interfere with maximum seroconversion). Mumps vaccine is also recommended for adolescents and adults who have not encountered mumps during childhood. Vaccine should not be administered to persons with febrile illness, congenital immunodeficiencies, leukemia, or lymphoma, or to those receiving immunosuppressive therapy. Because of the theoretical risk of fetal damage, vaccine is also not recommended for pregnant women.

Fig. 34-17. Incidence rate of mumps, United States, 1922–1983. (From Centers for Disease Control.)



Mumps vaccine is considered one of the safest of the childhood immunizing agents, yet only 31 states require proof of mumps immunity as a condition for entry into school. The incidence of mumps in states without a compulsory school mumps immunization law is twice as high as that in states with such a law.

RUBELLA

Rubella was first described in Germany in the 1800s and was subsequently called German measles. In 1941 Sir Norman Gregg suggested that rubella infection during the first trimester of pregnancy was responsible for crippling effects on the fetus (teratogenic effects), but the virus was not isolated until 1962. The full clinical implications of rubella infection were not realized until the pandemic of 1964, in which more than 30,000 infants were born with congenital abnormalities.

Rubella virus belongs to the family Togaviridae. It is a spherical virus 60 nm in diameter and consists of a nucleocapsid surrounded by a lipoprotein envelope. The virus also contains a hemagglutinin that interacts with erythrocytes from day-old chicks, geese, or pigeons. The virus can be cultivated on a large number of cell types, and isolation from clinical specimens is not difficult.

Pathogenesis

POSTNATAL RUBELLA INFECTION. Postnatal rubella infection is transmitted via the upper respiratory tract. The incubation period for the disease is 14–21 days. The virus multiplies in the mucosa of the respiratory tract and spreads to the bloodstream. During the viremic stage the virus is routinely found in the throat, blood, and feces. As the antibody level increases during infection, circulating virus decreases, a rash appears, and virus is found only in the nasopharynx. In addition to the rash, symptoms include leukopenia and suboccipital lymphadenopathy. The disease is self-limiting although complications such as arthritis and arthralgia are commonly encountered by young women.

CONGENITAL RUBELLA INFECTION. Congenital rubella results from infection of the fetus during the vi-

remic stage in the pregnant mother. The disease can follow infection during any trimester of pregnancy but is more prevalent when infection occurs in the first trimester. More than 30 percent of fetuses infected during the first trimester will be stillborn, aborted, or deformed at birth. Congenital anomalies of the infant may include cataracts and glaucoma, heart defects, deafness, and encephalitis. The virus is not lethal to fetal tissue but does cause inhibition of mitosis, which culminates in underdeveloped organs or tissue. The mortality for babies symptomatic at birth is 20 percent. Lymphocytes from children with congenital rubella syndrome exhibit chromosomal breaks.

Epidemiology

Rubella is worldwide in distribution and occurs more frequently during the winter and spring months. The virus is harbored in the upper respiratory tract of infected individuals and is transmitted by person-to-person contact. Both symptomatic and asymptomatic persons can be a source of infection. Patients are infectious 1–2 days before and 1–2 days after the appearance of the rash. In congenital rubella syndrome the virus can be shed for several months after delivery. This is a particularly important aspect of rubella infection because infant viral carriers are a constant source of infection to unimmunized nurses and other female personnel who come in contact with them.

The incidence of rubella and congenital rubella syndrome has steadily declined since 1969, the year rubella vaccine was first made available to the public (Fig. 34-18). Before 1969 approximately 50,000 cases per year were reported in the United States. In 1974 more than 62 percent of the children aged 1–12 years had received the rubella vaccine. In 1976 12,000 cases were reported. The number of cases of rubella reached an all-time low in 1983, when only 942 cases were reported. The incidence of rubella in adolescents and young adults did not decrease appreciably after the licensure of the vaccine until 1981. Approximately 10 to 20 percent of this group had been susceptible to rubella infection, but from 1981 to 1983 the incidence rate for adolescents and young adults was lower than that for young children. Concurrently, there has also been a drop in the number of cases of congenital rubella. Fifty-five cases were reported in 1979, fourteen in 1980, and only three in 1983.

Laboratory Diagnosis

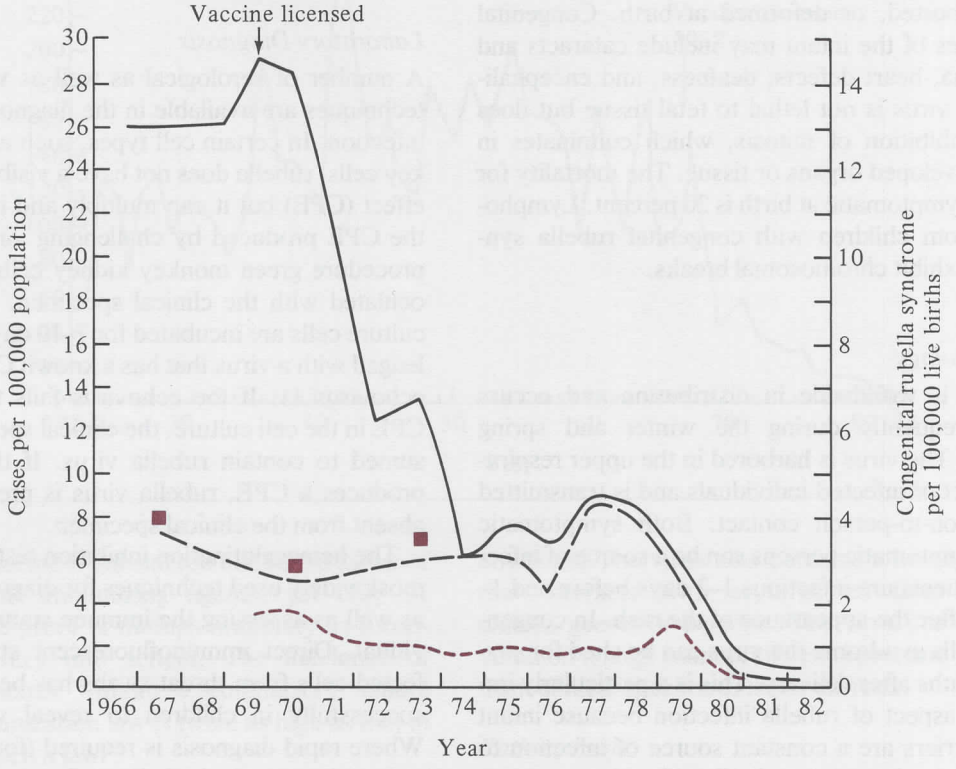
A number of serological as well as viral isolation techniques are available in the diagnosis of rubella infection. In certain cell types, such as green monkey cells, rubella does not have a visible cytopathic effect (CPE) but it can multiply and interfere with the CPE produced by challenging viruses. In one procedure green monkey kidney cells are first inoculated with the clinical specimen. The infected culture cells are incubated for 7–10 days, then challenged with a virus that has a known CPE—such as echovirus 11. If the echovirus fails to produce a CPE in the cell culture, the clinical specimen is presumed to contain rubella virus. If the echovirus produces a CPE, rubella virus is presumed to be absent from the clinical specimen.

The hemagglutination inhibition test is one of the most widely used techniques for diagnosing rubella as well as assessing the immune status of the individual. Direct immunofluorescent staining of infected cells from throat swabs has been employed successfully in children to reveal viral antigen. Where rapid diagnosis is required (for example, in pregnant women), the indirect fluorescent antibody technique is recommended. In this test rubella-specific IgM antibody can be quickly detected in the patient's serum. Most rubella antibody testing in the United States is now done with commercially prepared test kits. Passive hemagglutination and latex agglutination are two types of tests available with these kits.

Immunity

Immunity to rubella infection is apparently lifelong, but not enough years have elapsed since licensure of the vaccine to determine the effect of vaccination. Recent studies have indicated that vaccine-induced antibody persists up to 16 years in the majority of those vaccinated. However, up to 10 percent of those vaccinated have low levels of antibody 6

Fig. 34-18. Incidence rate of reported rubella and congenital rubella cases, United States, 1966–1982. (— = total rubella; •—•—• = rubella in those 15 years or older; — — — = congenital rubella; ■ = average annual United States estimates of rubella incidence in those 15 years or older based on data from Illinois, Massachusetts, and New York City for period from 1966–1974.) (From *MMWR* 32:505, 1983.)



years after vaccination. Subclinical infection can take place with wild rubella virus, but such infections are associated with those who have vaccine-induced immunity. The virus can be isolated from the upper respiratory tract in subclinical infections, but the period of virus shedding is short and the risk of viremia is not evident. Viremia may be possible in those with vaccine-induced immunity. Susceptible contacts are not believed to be at risk, and immune women reinfected are unlikely to infect their fetuses.

Prevention and Treatment

There is no treatment for either congenital or postnatal rubella infection. If infection occurs during the first trimester of pregnancy, therapeutic abortion is recommended by many doctors. Control of rubella rests primarily on vaccination procedures aimed at protecting women of childbearing age. Immunization in the United States is advised for children between the age of 1 year and puberty, and for adolescent girls and adult women who are seronegative and in whom pregnancy can be avoided at least 3 months after vaccination. Although the risk of rubella may be minor, pregnant women can become infected and should avoid contact with rubella patients.

An attenuated rubella vaccine (RA 27/3) developed in 1979 has replaced older vaccines (Cendehill or HPV-77) for use in the United States. The vac-

cine may be given intranasally or by injection. It is used either in monovalent form or in combination with measles or measles-mumps vaccines. The trivalent vaccine is recommended in infant vaccination programs but should be given at 15 months of age to achieve maximum measles seroconversion.

No evidence exists that vaccination after exposure is protective, but it should be carried out in case of future exposure. Immune serum globulin (ISG) administered after exposure will not prevent infection but may suppress or modify symptoms. ISG as a postexposure prophylaxis in early pregnancy is not advocated. Vaccine should not be administered to pregnant women because of the risk of fetal abnormalities, since the vaccine virus can cross the placenta. Rubella vaccination of a pregnant woman does not in itself indicate interruption of pregnancy. Patients with suppressed immune responses should not receive the vaccine.

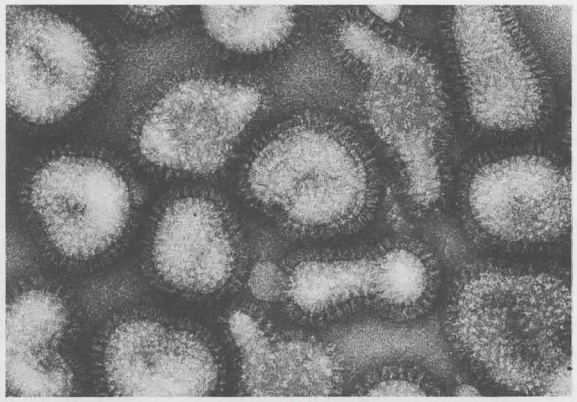
Vaccine side effects are more severe in adults, particularly women, than in children. Arthralgia and transient arthritis are the major complaints, but the symptoms seldom last more than 2–3 weeks. Recurrences of arthritis, especially in the knee joint, can prevail up to 24 months after vaccination.

The periodic outbreaks of rubella among office personnel and, even more important, hospital personnel, put pregnant women at risk (see boxed essay, p. 738). It is estimated that 20 to 30 percent of women of childbearing age lack rubella antibody. Recommendations by hospital advisory committees on immunization of hospital personnel who have contact with female patients of childbearing age have not always been followed. Currently, only four states have laws requiring proof of immunity to rubella for hospital personnel.

INFLUENZA

The influenza viruses belong to the family of RNA viruses called the Orthomyxoviridae. They are usually spherical, although filamentous forms are known, and measure 80 to 100 nm in diameter (Fig. 34-19). Surrounding the internal ribonucleoprotein

Fig. 34-19. Electron micrograph (× 180,000 before 25% reduction) of human influenza virus. (Courtesy R. C. Williams.)



is a lipid envelope from which project hemagglutinin and neuraminidase peplomers (see Fig. 33-14). Agglutination of red blood cells by influenza virus is initiated by the interaction of glycoprotein receptors on the erythrocytes and the hemagglutinin peplomers of the virus. This causes an agglutination of the erythrocyte-virus aggregate. The glycoprotein receptor on the red blood cell also serves as the substrate for the other peplomer, neuraminidase. The enzyme causes the release of the virus from the red blood cell. The dissociated erythrocytes are no longer agglutinable by homologous viruses.

The RNA genome of the influenza virus is unique among RNA viruses in that it consists of eight distinct RNA molecules that may be linked as a complete unit only in the mature virion. The presence of these distinct RNA units during replication in the animal host explains the high rate of genetic recombination observed when cells are infected with different strains of influenza virus. Recombination is the genetic mechanism responsible for the major antigenic differences in pandemics caused by the influenza virus.

The virus is stable to freezing and thawing but is inactivated by heat and ultraviolet light.

RUBELLA OUTBREAK AMONG OFFICE WORKERS—NEW YORK CITY

From February 2 through May 13, 1983, 86 cases of clinical rubella were reported among office workers employed at a major bank in lower Manhattan. The 86 employees ranged in age from 18 to 54 years. The source of the outbreak was not identified.

Three serologically confirmed cases occurred in pregnant women; infection occurred at 14, 18, and 28 weeks' gestation. None of these women had a history of rubella vaccination. One woman subsequently delivered a full-term infant with congenital anomalies, but the cause of the anomalies is not certain. The other two pregnancies have not yet reached term.

Of the 86 patients, 72 (83.7%) worked in one of three affected office buildings; of these, 56 (77.8%) were women. The attack rate was significantly higher for women (2.1%) than for men (0.8%). Also, the risk of infection was significantly higher for women of childbearing age, under 45 years old (2.6%), than for women 45 years old or over (0.8%). However, work location was the most important risk factor. Of 72 ill persons in building A, 40 worked in offices on the eleventh through fourteenth floors. Employees on these floors were 3.8 times more likely to have rubella than office workers on other floors. Women of childbearing age who worked on the eleventh through fourteenth floors, the group with the highest attack rate, were 4.8 times more likely to have rubella than women of childbearing age who worked on other floors.

When rubella was first suspected, employees were advised to report all rash illnesses to the employee health unit. In addition, susceptible women of childbearing age were urged to be vaccinated unless pregnant, and pregnant women were advised to stay home until their immune status was known. After rubella infection was serologically confirmed, the Department of Health established an immunization clinic in the office building from May 4 to May 9. Of 6409 employees working in the complex, 2362 (36.9%) were vaccinated. Women of childbearing age were counseled regarding the theoretical risk to the fetus from vaccination and the importance of avoiding pregnancy for 3 months after vaccination. Before vaccination, serum specimens were collected from approximately 1000 women of childbearing age. The sera were stored for antibody testing for any woman who might become pregnant within 3 months after vaccination. Also, a surveillance system was established to identify pregnancies or adverse events after vaccination. As of May 23, one pregnancy in a susceptible woman and adverse events for six persons were reported. The adverse events included lymphadenopathy (four persons), joint pain (three), diarrhea (one), dizziness (one), and sore throat (one).

From *MMWR* 32:349, 1983.

Classification

There are three types of influenza viruses: *A*, *B*, and *C*. Types B and C have been isolated only from humans, while type A has been found in horses, swine, and birds as well as humans. The separation of these viruses into types is based on differences in the antigenicity of their ribonucleoprotein and M protein, which is part of the inner surface of the lipid layer of the envelope (see Fig. 33-14). Strain specificity resides in the peplomers (that is, hemagglutinin and neuraminidase). Four types of hemagglutinin (H0, H1, H2, H3) and two types of neuraminidase (N1, N2) are recognized among influenza A strains causing illness in humans. These antigens are the basis for classifying new strains. The classification items used to describe, for example, the strain A/duck/Tokyo/5/69/(H2N2) of influenza A are as follows:

1. Antigenic type of nucleocapsid: A, B, or C; for example, influenza A
2. Source of the isolate if from a species other than humans: A/duck
3. Geographical location or origin of the isolate: A/duck/Tokyo
4. Strain number: A/duck/Tokyo/5
5. Year of isolation: A/duck/Tokyo/5/69
6. In parentheses, the antigenic identity of the hemagglutinin (H) subtype and neuraminidase (N) subtype: A/duck/Tokyo/5/69/(H2N2) (The numbers after the hemagglutinin and neuraminidase subtypes [H2N2] are antigenic variants of earlier strains.)

Pathogenesis

Influenza is a disease of the upper respiratory tract. Infection is acquired primarily by inhalation of aerosols produced by infected individuals. Influenza is usually a benign disease characterized by no symptoms or very mild symptoms. Infecting viral particles attached to the respiratory epithelium may be neutralized by antibodies in the mucosa or by glycoprotein inhibitors, which prevents the attachment of virus to the epithelial surface. If infection does take place, viral neuraminidase is believed to lower the viscosity of the mucus in the respiratory tract and permit the attachment of virus to epithelial cell receptors. In uncomplicated infections the virus does not penetrate into the lower respiratory tract but remains in the upper or middle tract, resulting in necrosis and desquamation of the respiratory epithelium. In complicated cases the virus infects the epithelial tissue of the lungs, causing viral pneumonia, which can lead to secondary bacterial infections.

The incubation period for influenza is 24–48 hours; it is usually followed by fever, cough, headache, muscular aches, sore throat, and conjunctivitis. Acute symptoms last for approximately 3–5 days. About 2 percent of persons infected have secondary bacterial pneumonia caused as a rule by *Staphylococcus aureus*. Encephalitis and meningitis are also complications of influenza virus infection.

A complication of influenza A and B infections as well as varicella is called *Reye's syndrome*. The syndrome occurs in children between the ages of 6 and 11 who are recovering from infection by these viruses. Manifestations of the disease include intractable vomiting, lethargy, and delirium. Untreated, the disease may progress to coma and death. Brain damage has been reported in some survivors, but most recover without affliction. Several studies have suggested that treatment of respiratory infections with salicylates to reduce fever may be a contributing factor to the syndrome. One of the features of Reye's syndrome is injury to mitochondria, especially hepatic mitochondria, which become disrupted. This characteristic is verified by the elevated levels of mitochondrial enzymes in the serum as well as elevated amounts of ammonia, lactate, pyruvate, alanine, and free fatty acids. These products are produced in part by reactions localized in the mitochondria. Metabolites such as ammonia and fatty acids are toxic to the central nervous system and thus could be responsible for encephalopathy. The risk of Reye's syndrome appears to be highest after influenza B infection, followed by varicella-zoster and influenza A.

Epidemiology

Influenza is an endemic disease in which epidemics take place every 2–5 years. The pandemics that occur approximately every 8–10 years are caused by influenza A virus. The first human influenza virus was isolated in 1934 and was the prototype, designated influenza A (A0). In the 1957 influenza pandemic, the infecting isolate was placed in a new serotype called Asian influenza (A2). Other than an antigenic relationship to the nucleocapsid, the A2 isolate exhibited no antigenic similarities to any of the influenza viruses isolated from 1933 to 1956. The Asian isolate has been shown to be related to swine, fowl, and horse influenza viruses in addition to human strains, but it is unrelated to influenza B or C viruses.

In the 1957 pandemic more than 80 million cases of influenza were recorded. In the United States alone, an estimated 88,000 deaths were pneumonia-influenza related. Most deaths were among the elderly and people seriously debilitated with chronic cardiovascular and lung diseases.

In 1968 an outbreak of influenza occurred in Hong Kong and spread throughout the world. In the United States nearly 20,000 deaths were attributed to the Hong Kong strain. A new antigenic subtype H3N2 of influenza A was responsible for this pandemic, and it remained the prevalent antigenic type until 1978. In 1976 a new type A influenza virus that was similar to swine influenza (Hsw1N1) appeared in Fort Dix, New Jersey. Many epidemiologists feared that a major epidemic would soon follow, and mass vaccination was initiated. The epidemic, however, never materialized.

In 1977 the Soviet Union reported large-scale outbreaks of influenza caused by viruses antigenically related to H1N1. H1N1 viruses had been a source of disease in the Soviet Union and China from 1947 to 1956. The prototype was designated A/USSR/90/77/(H1N1), and this strain has replaced H3N2 viruses in China and the Soviet Union as the major strain causing disease. In the United States H3N2 strains are still responsible for approximately 80 percent of the cases of influenza, while H1N1 strains account for 10 percent, and 10 percent are caused by type B isolates. H3N2 strains are nearly always associated with outbreaks in hospital settings or nursing homes, while H1N1 strains are more frequently associated with outbreaks in schools.

Epidemics caused by influenza B viruses are less frequent than those caused by influenza A. Influenza B viruses show more antigenic stability and are more easily handled in epidemics. Strains of influenza isolated in the United States in 1978 were similar to the B/Hong Kong/5/72 virus. Influenza B appears in the United States more frequently in local epidemics. Vaccines containing the B type antigens are available.

Type C influenza is prevalent throughout the world, and infection occurs early in life. Outbreaks caused by type C virus are rarely reported and are usually discovered retrospectively by serological studies associated with other types of influenza. Thus little is known of the pathogenesis or epidemiology of the type C influenza.

Between major epidemics, antigenic changes take place in the virus. Minor antigenic changes (*antigenic shift*) result in influenza infections in which antibodies in the population have a low affinity for the virus. Major antigenic changes (*antigenic drift*) result in influenza infections in which there are few or no antibodies against the virus. Lack of antibodies to a new strain in which major antigenic changes have taken place can result in pandemics. As the population develops an immunity to the new strain there are likely to be periodic small epidemics among those who did not come in contact with the new strain and thus did not develop an immunity against it (Fig. 34-20). Antigenic drift is believed to result from a genetic recombination process involving the different segments of the influenza virus genome.

Laboratory Diagnosis

Diagnosis of influenza is based on isolation and identification of the virus or by observation of changes in the antibody titer of affected individuals. Throat and nasal specimens (swab or washings) are first treated with antibiotics and then clarified by centrifugation before inoculation into

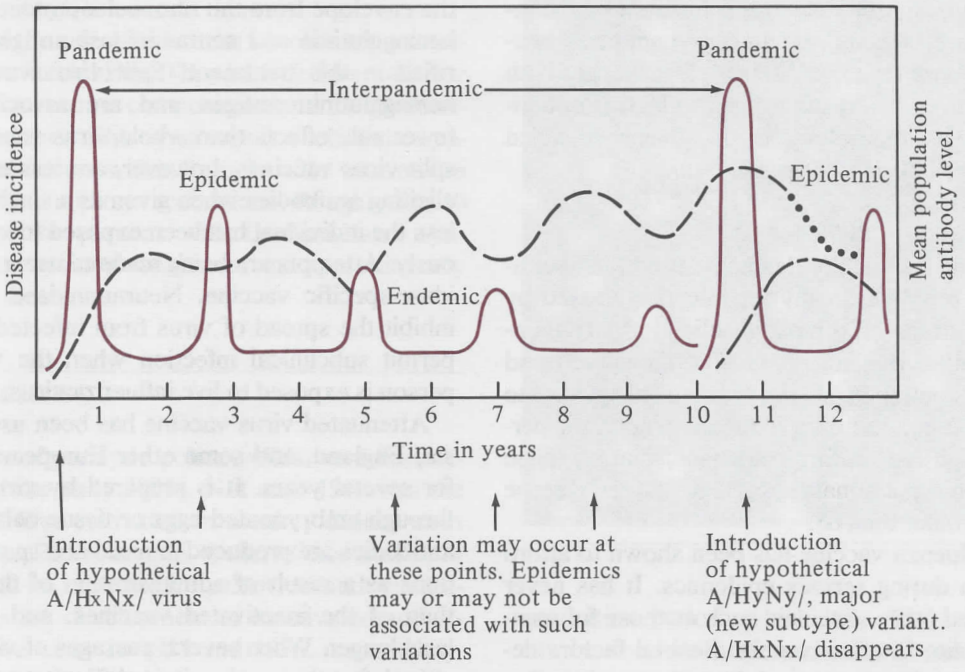


Fig. 34-20. Scheme for occurrence of influenza pandemics and epidemics in relation to level of immunity in the population. (— = incidence of clinically manifest influenza; — — — — = mean level of population antibody against A/HxNx/; —•—•— = mean level of population antibody against A/HyNy/.) (From R. G. Douglas. In G. J. Galasso, T. C. Merigan, and R. C. Buchanan [Eds.], *Antiviral Agents and Viral Diseases of Man*. New York: Raven, 1979.)

embryonated eggs or cell culture. After approximately 5 days of incubation the cultivated virus can be identified by hemagglutination using chick or guinea pig erythrocytes. Hemagglutination inhibition, neutralization, and complement fixation tests can also be employed for identification. In tissue culture, such as monkey kidney cells, presumptive identification of influenza B can be made because of the virus's characteristic cytopathic effect. Typing of the influenza virus into A, B, or C can be made by identification of the ribonucleoprotein (soluble antigen) in a double immunodiffusion test.

Serological diagnosis is based on a fourfold rise in antibody titer between the acute and convalescent sera as determined by the complement fixation test. During suspected epidemics, presumptive diagnosis is made by comparing the sera of recovered influenza patients and of those in the acute phase of the illness. Sera from both groups are tested for A and B ribonucleoproteins. If an epidemic has been caused by the influenza virus, the antibody titer to the A or B antigens should be higher in sera from convalescents than from the acutely ill.

Immunity

After infection, immunity lasts for only 1 or 2 years. Reinfection depends on the antigenic characteristics of the infecting virus strain and the patient's prior contact with related strains. Resistance to one strain does not always confer resistance to other strains. A person's serological response to in-

fluenza A is dominated throughout life by the type of antibody produced as a result of his or her first experience with the virus. A person challenged with one antigen and then rechallenged with an influenza virus containing related and unrelated antigens will exhibit two immunological responses: First, an anamnestic response is initiated by the related antigen. Second, the unrelated antigen stimulates a primary response. Repeated challenges with related antigen will result in the production of antibodies against the first strain. This has been called the *doctrine of original antigenic sin*.

Prevention and Treatment

Amantadine hydrochloride can be used therapeutically and prophylactically for infection caused by all type A strains. It is most beneficial when administered within the first 2 days of the illness and should be continued as long as there is exposure to virus. This antiviral drug is recommended for persons at high risk during epidemics, that is, those with chronic pulmonary, heart, or kidney disease and those older than 65.

The influenza vaccine has been shown to afford protection during serious epidemics. It has never approached other vaccines, such as those for measles or polio, in effectiveness. Several factors determine the effectiveness of the influenza vaccine: (1) method of preparation of vaccine, (2) concentration of virus in the vaccine, (3) route of inoculation, and (4) previous exposure of the vaccinated person to the strain of virus being used in the preparation.

The most widely used commercial preparation is the formaldehyde-inactivated vaccine. This is prepared from infected chick embryo and consists of serotype strains of influenza virus type A and influenza virus type B. One of the major difficulties encountered with the inactivated vaccine is the incidence of systemic reactions after vaccination. Approximately 4 percent of persons 6 years old or older are subject to these responses. Vaccines prepared by zonal centrifugation are purer and cause fewer postvaccination responses. The vaccine is administered subcutaneously; however, many investigators feel that intranasal administration followed by booster doses is more practical since intranasal IgA plays a more direct role in immunity than do serum antibodies.

Subunit (split virus) vaccines have been produced by treating virus with polysorbate (Tween) and ether, sodium deoxycholate, or tri-N-butyl phosphate. The treatment results in the release of the envelope from the ribonucleoprotein core. The hemagglutinin and neuraminidase antigens are purified in this treatment. Split virus vaccines use hemagglutinin antigen and are associated with fewer side effects than whole virus vaccines. The split virus vaccines, however, are less effective in eliciting antibodies when given as a single dose unless the individual has been exposed to virus previously. Attempts are being made to use a neuraminidase-specific vaccine. Neuraminidase antibodies inhibit the spread of virus from infected cells and permit subclinical infection when the vaccinated person is exposed to live influenza virus.

Attenuated virus vaccine has been used in Russia, England, and some other European countries for several years. It is prepared by virus passage through embryonated eggs or tissue culture. More antibodies are produced in nasal and sputum secretions as a result of administration of this vaccine than of the inactivated vaccines, and protection lasts longer. When several passages of virus are required for attenuation it is difficult to retain any substantial level of immunogenicity.

A major problem with influenza virus vaccine preparation is that the hemagglutinin antigen that induces protective antibodies is constantly changing (antigenic drift). Close observation of the prevalent infecting influenza virus during epidemics and pandemics is therefore important so that effective viral antigens can be used in vaccine preparations.

It is generally recommended that only people who are most likely to be at risk be vaccinated: the aged, those debilitated by pulmonary and cardiac diseases, those with diabetes mellitus or other metabolic diseases, those with chronic renal disease, and those with conditions that compromise the immune mechanism. The influenza vaccines for 1983–1984 include hemagglutinin of A/Brazil/78(H1N1), A/Philippines/82(H3N2), and B/Singapore/79 viruses. Split virus is used for children from

3 months to 12 years of age, while whole or split virus can be administered to those over 12 years of age. A single dose is administered, followed by a booster dose in 2 months. For those who receive yearly vaccines, boosters are not advised unless the antigenic characteristics of the virus have changed enough to warrant them.

Most of the side effects from vaccine administration appear in children. They include fever, allergic response to vaccine components, and the *Guillain-Barré syndrome*. This syndrome is a self-limited paralysis that occurs within 8 weeks after vaccination in 10 of every million vaccinations. Roughly 5 to 10 percent of the persons with Guillain-Barré syndrome have residual weakness, and approximately 5 percent die.

POLIOMYELITIS

Epidemics of paralytic poliomyelitis were first described in the early 1800s. Not until 1949 was the poliovirus first cultivated in monkey kidney cells by Enders, Robbins, and Weller. Once sufficient virus could be cultivated, further research eventually led to the development of inactivated and attenuated vaccines in 1955 and 1956, respectively. Because of these vaccines, poliomyelitis in the developed countries of the world is almost a clinical oddity.

The poliovirus belongs to the family Picornaviridae, and is grouped in the genus enterovirus. It is a spherical, nonenveloped RNA virus (Fig. 34-21) that measures approximately 25 to 30 nm in diameter. Three distinct serotypes can be distinguished in the neutralization test.

Pathogenesis

In more than 99 percent of persons infected with the poliovirus, clinical symptoms are very mild or inapparent. The virus is, under most circumstances, taken into the mouth through contaminated food, water, or milk. It multiplies in the nasopharynx or intestinal mucosa of the small intestine and from these sites of multiplication can be disseminated via the bloodstream. During the incubation period, which lasts 10–15 days, virus can be shed in the feces and will continue to be shed despite high titers of humoral antibodies. At the onset of illness the patient has fever, headache, and stiffness in the neck. In nonparalytic polio these symptoms subside in a few days and recovery is complete. In about one percent of the cases paralysis occurs. Paralysis is the result of invasion of the CNS by the virus from the bloodstream or along the axons of peripheral nerves to the anterior horn cells of the spinal cord. The virus may also affect the posterior horn and dorsal root ganglia. Multiplication of the virus in the neurons causes their destruction; as a result, nerve cells innervating the voluntary muscles are no longer functional and the muscles atrophy. Full or partial restoration of muscle function depends on the number of uninjured neurons that remain to innervate the muscle and the ability of unaffected muscles to hypertrophy and assume motor function.

Involvement of the CNS during infection by the

Fig. 34-21. Electron micrograph of poliovirus. (From J. L. Melnick, *Intervirology* 20:61, 1983.)

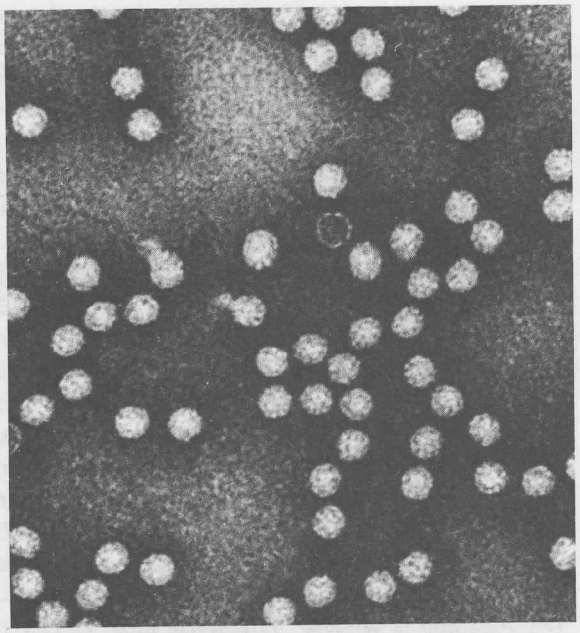
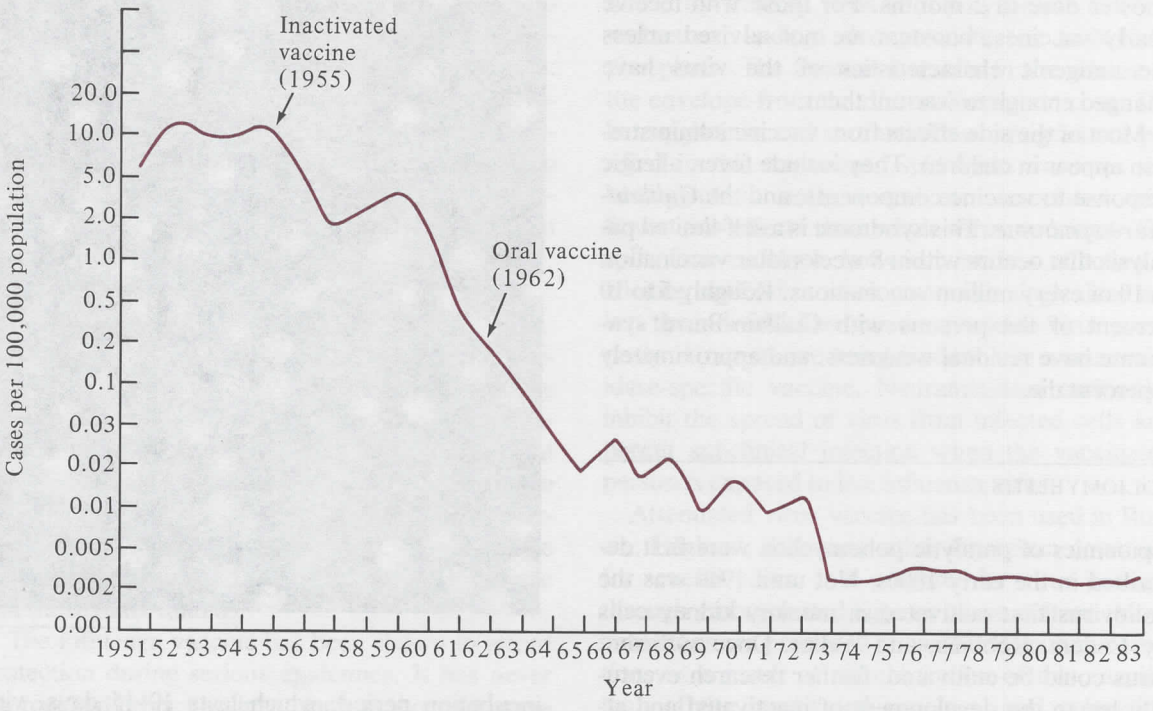


Fig. 34-22. Reported paralytic poliovirus attack rates by year, United States, 1951–1983. (From Centers for Disease Control.)



virus may be enhanced under certain predisposing conditions such as tonsillectomy, pregnancy, steroids, fatigue, or conditions that may introduce virus into peripheral nerve fibers.

Epidemiology

Poliomyelitis is a human disease caused by three serotypes of poliovirus. Most infections in the United States have been caused by type 1. Permanent immunity is acquired only to the serotype causing the infection. Poliovirus is spread by the fecal-oral route and can infect any age group. Paralytic cases occur primarily in young adults and adolescents. Infection in the very young and infants often produces an asymptomatic disease that brings complete immunity. It is a paradox that before the discovery of polio vaccines improvement in sanitation practices had actually increased the risk of paralytic polio. Before improvements in sanitation the poliovirus was transmitted to infants through contaminated feces, milk, or water. Infection in the infant invariably resulted in asymptomatic disease that provided permanent immunity. As sanitation practices improved, many of the unvaccinated were not exposed to the virus until adolescence or adulthood, the period when infection most often causes paralysis or death.

Since the introduction of the inactivated vaccine in 1955 the number of cases of paralytic polio has decreased from 18,308 in 1954 to 8 in 1983 (Fig. 34-22). Polio is still very active in tropical and semitropical countries, occurring in 3-year cycles. In 1979 over 37,000 cases were reported to the World Health Organization, but it is estimated that only one-fifth to one-tenth of all cases are reported. Unfortunately, even with the availability of the polio vaccine, many countries do not have the budget or personnel to vaccinate all the people who require

vaccination. Some groups, for religious reasons, refuse to be vaccinated, and because of this epidemics occasionally occur. In 1971 28 families in a small village in the Netherlands became affected by poliovirus infection. Thirty-nine patients had neurologic complications: 28 with paralytic disease and 11 with meningitis. Five patients died.

Laboratory Diagnosis

Isolation of the virus is the preferred method of diagnosis. In the laboratory, clinical specimens, usually fecal, are prepared in suspension and treated with penicillin and streptomycin to inhibit bacterial growth. The suspension is then clarified by centrifugation. Aliquots of the viral suspension are inoculated into tissue culture tubes containing monkey kidney or human cells. The culture tubes are observed daily for CPE (that is, rounding and shrinking of cells). Presumptive diagnosis of poliomyelitis is made from the clinical history of the patient and the CPE produced in tissue culture. Since other enteroviruses may have similar effects, identification of poliovirus rests on neutralization of the cultivated viral agent by antipoliomyelitis serum.

Serological diagnosis of infection can be made by using paired sera, one collected at the onset of illness and the other 2–4 weeks later. The neutralization test is the preferred technique for determining the antibody titer against all three poliovirus types.

Prevention and Treatment

Since 1955 two vaccines have been employed in the prevention of poliomyelitis infection: an inactivated polio vaccine (IPV) and a trivalent oral polio vaccine (OPV). IPV was licensed in 1955; OPV in 1963. Both vaccines produce immunity in more than 90 percent of the recipients, but OPV has a higher benefit-to-risk ratio. IPV, which contains types 1, 2, and 3 poliovirus, is administered by injection and can be given simultaneously with diphtheria, tetanus, and pertussis antigen (DTP). Recipients can be infected with and become intestinal carriers of wild poliovirus or attenuated virus vaccine strains. For primary vaccination of children in the United States, OPV is the vaccine of choice because (1) it multiplies in the intestinal tract and establishes intestinal immunity to reinfection, thus breaking the chain of transmission; (2) it is simple to administer; (3) it does not require periodic boosters; (4) it is well accepted by patients; and (5) it has a record of having eliminated disease associated with wild poliovirus.

OPV is administered in three doses; the first two are given not less than 6 weeks apart, and the third dose should follow in 8–12 months. For infants the first dose is often given with the first dose of DTP. On entrance to kindergarten a single booster dose should be administered. Routine vaccination of adults in the United States is not necessary; the risk is small unless one is traveling to another country. Oral polio vaccine poses some risks. The vaccine contains live virus and is able to cause disease in those exposed either by active immunization or by contact with a vaccinated person. Most vaccinated persons who acquire poliomyelitis are less than 4 years of age, whereas those who acquire disease after contact with a vaccinated person are over 20 years of age. The risk of vaccine-associated paralysis from attenuated vaccine is one case per 15 to 20 million vaccinations. If there is a risk for the vaccinated person, for example, those with immunodeficiency, the killed vaccine can be substituted for the attenuated vaccine. Another disadvantage with the attenuated vaccine is the necessity of maintaining cool temperatures. The oral vaccine is very susceptible to heat. At 20–25°C inactivation occurs in less than 2 weeks; at temperatures greater than 37°C, in 1–3 days. Vaccine can be stored safely for 6 months under refrigeration.

Although the risk of poliomyelitis is small, recent studies show over 30 percent of children 1–4 years old have not had primary vaccination. Because inapparent infections caused by wild strains of poliovirus have diminished, the risk of poliomyelitis in children is greatly increased. It is very important that parents have their children immunized as recommended.

RABIES

Rabies is an infectious viral disease that affects the CNS. Even before the discovery of viruses, Pas-

Fig. 34-23. Electron micrograph of a purified preparation of rhabdovirus particles. Note the bullet shape of the particles. (From R. W. Horne, *Virus Structure*. New York: Academic, 1974.)

teur recognized in the 1880s that the causal agent of rabies was not a bacterium but a submicroscopic microorganism. He prepared the first rabies vaccine by intracerebral passage of the infectious agent in rabbits. The vaccine was first administered to a French peasant boy who was later to become the gatekeeper at the Pasteur Institute in Paris. In 1904 Negri made the first histological diagnosis of rabies when he observed inclusion bodies in the cytoplasm of nervous tissue. These inclusion bodies bear his name: *Negri bodies*.

The rabies virus is an RNA virus belonging to the family Rhabdoviridae. It is bullet shaped and measures 180 × 75 nm (Fig. 34-23). A lipoprotein envelope encompasses the nucleocapsid and contains glycoprotein peplomers that constitute the hemagglutinins of the virus.

The rabies virus can infect most warm-blooded animals, including humans. In the laboratory the virus can be cultivated in chick embryos, hamster kidney cells, and human diploid cells. Wild virus isolated from nature is termed *street virus*. When wild virus is attenuated in the laboratory it is referred to as *fixed virus*. Fixed virus has a higher infectivity titer and shorter incubation period than street virus.

Pathogenesis

Rabies is usually transmitted to people through the bite of infected animals. There are cases of infection by aerosols created by infected bats. The incubation period for the disease is long and variable, depending on the site of infection, the infectious dose of virus, and the length of time required for the virus to migrate to the spinal cord or brain. The incubation period in humans varies from 2 to 16 weeks but in a few cases has lasted 2 or 3 years. The virus is propagated along peripheral nerves of the CNS, from which it migrates to other tissues such as the salivary glands. Viremia has not yet been adequately demonstrated and plays no role in pathogenesis. In the brain the virus causes severe nerve cell damage. Demyelination occurs in the white matter.

The first symptoms of rabies include fever, changes in temperament, and coryza (common cold symptoms of sneezing and watery eyes). Within a few days the patient may become excitable and exhibit photophobia, difficulty in swallowing, drooling, and fear of water (hydrophobia). During the last stages before death the patient develops convulsive seizures and lapses into a coma.

Epidemiology

Rabies has been reported from every continent except Australia and Antarctica. The disease is endemic in areas of Asia, Africa, and both North and South America. In nature the virus's permanent host is the skunk, weasel, or mongoose. The virus is maintained in wildlife because of its ability to invade and replicate in various tissues and organs

such as kidneys, lungs, and salivary glands without invading neural tissue and causing encephalitis. Various wild animals are a source of infection for domestic animals such as dogs and cats and even occasionally cattle and horses. Domestic dogs are still the most important source of human disease in areas where domestic animal rabies has not been controlled by animal vaccination. In those areas where domestic animals have been vaccinated most cases of human rabies result from wild animal bites or from imported cases of rabies. In the United States human rabies has been associated with rabies among animals such as skunks, raccoons, foxes, and bats. In the southeastern states raccoons are the major rabies reservoir. The problem has been exacerbated by the practice of some individuals of importing raccoons from various states for the benefit of raccoon hunters. Rabies virus may be present in the saliva of skunks for up to 14 days before the signs of disease appear. More virus accumulates in skunk saliva than in that of either the dog or fox. The saliva is believed to be infective in only 50 to 60 percent of infected dogs at the time of the bite. Virus can multiply in the nasal mucosa of infected bats and is shed as an aerosol, thus presenting an occupational hazard to speleologists.

Since 1960 fewer than three cases of rabies have occurred in the United States per year; however, more than 35,000 people a year are given postexposure rabies vaccine (see boxed essay, p. 748). Human-to-human rabies transmission by corneal transplants has been reported on four occasions. The diagnosis of rabies in all four cases was not suspected before the donor's death.

Laboratory Diagnosis

Microscopic examination of brain tissue for rabies virus is the most common laboratory diagnostic technique. The fluorescent antibody test provides a rapid diagnostic procedure and has supplanted other tests such as Negri body determination and isolation of virus by mouse inoculation.

When a person is bitten by a dog or other animal the latter is placed in quarantine for 7–14 days. If the animal is without symptoms it is released, but if it develops symptoms of rabies, its brain tissue is examined for rabies virus by fluorescent antibody or the tissue is examined for the appearance of Negri bodies. If these tests are not conclusive a suspension of brain or submaxillary gland tissue is inoculated intracranially into test animals such as suckling hamsters, rabbits, or mice. The experimental animals are later examined for the appearance of Negri bodies and rabies antigen by the fluorescent antibody test, as well as any clinical symptoms suggestive of rabies.

In humans diagnosis of rabies is dependent on clinical illness and epidemiological evidence of potential rabies exposure. Serological techniques are of little value since antibody does not appear until the eighth day of the illness. Sometimes viral antigen can be detected in smears prepared from corneal or nasal swabs by the direct fluorescent antibody technique. High levels of antibody in the cerebrospinal fluid (CSF) can be accepted as diagnostic of rabies even after administration of vaccine. Clinical rabies, but not vaccine, can stimulate production of antibodies in the CSF.

Immunity

The fact that antibody is not present in the infected individual until the eighth day of illness indicates that rabies does not stimulate the host immune response until the virus reaches the brain. A person receiving no therapy within this period will die before his or her own immune system can respond. Only three persons with clinical rabies have ever been known to survive.

Prevention and Treatment

Although rabies is rare in the United States, the management and treatment of persons infected is extremely important. The Public Health Service recommends that each exposure to possible rabies infection be evaluated individually. Thus the attending physician must take into account the animal initiating the attack, the epidemiology of rabies in the community, the circumstances under which the attack occurred, and the vaccination status of the attacking animal.

After attack by a rabid animal or one suspected

HUMAN RABIES—MICHIGAN

The first case of human rabies acquired in the United States since May 1981 occurred recently in Michigan. The patient, a 5-year-old girl possibly bitten by a bat in late August 1982, died on March 9, 1983, 32 days after onset of symptoms.

The child developed right arm pain and fever after a fall. On February 7, an acute sprain of the right arm was diagnosed. By February 11, she had malaise, anorexia, sore throat, left heel pain, and right arm weakness. She appeared ill but was alert and cooperative after hospitalization, and had point tenderness at her right wrist, elbow, and shoulder and left heel. The white blood cell (WBC) count was 12,800 and a bone scan of the right wrist showed evidence of osteomyelitis. Over the next 48 hours, she became irritable with temperatures to 103.9°F, progressive right arm weakness, urinary incontinence, and difficulty swallowing saliva and water.

On February 13 she became lethargic and hypertensive, and was transferred to another hospital. Rabies was considered but no clear history of animal exposure could be obtained. Cerebrospinal fluid (CSF) revealed 10 WBC and negative bacterial cultures. Computed tomography and brain scans were normal, and the electroencephalograph was diffusely abnormal without focal findings. The next day, she became obtunded and developed progressive respiratory distress requiring mechanical ventilation. The presumptive diagnosis was postinfectious encephalopathy, and treatment with high-dose steroids was initiated. By February 17 she was comatose.

On February 23, the family remembered a possible bat bite in late August, 1982. Sera collected on February 23, 18 days after the onset of symptoms, showed high titers of rabies antibody to rapid fluorescent focus inhibition test (RFFIT) and by immunoadherence hemagglutination (AHA) at the Michigan Department of Public Health. Antibody was not present in the CSF. Since skin biopsy and mouse inoculation were negative, the serum results were not considered sufficient to confirm the diagnosis of rabies. Sera and CSF collected around February 28 showed no titer rise, but on March 4 the serum and CSF showed rises to 1:25 and 1:17, respectively, by RFFIT. A presumptive diagnosis of rabies was made. On March 9, the patient had a cardiac arrest and died. The Michigan Department of Public Health identified rabies virus from the brain by direct fluorescent antibody. Of 254 persons at the two hospitals who had contact with potentially infectious secretions from the patient, 54 received postexposure prophylaxis.

Without treatment, rabies antibody titers typically rise to levels of 1:10,000 to 1:60,000. Concurrent steroid therapy has been shown to prevent antibody formation in rabies vaccine recipients and, like interferon, appears to have little effect on clinical illness.

From *MMWR* 32:159, 1983.

of being rabid, the bite wound should be cleansed by flushing with soap and water followed by application of 40 to 70 percent alcohol, tincture of iodine, or 0.1 percent quaternary ammonium compounds. Vaccine and immune serum are administered according to the schedule indicated in the next paragraph. During the hospitalization of the patient, many complications involving vital functions may occur. Pulmonary and cardiovascular problems are common, and these vital systems must be monitored continually. Sedation of the patient is imperative if seizures are to be controlled. Such procedures protect not only the patient but attending hospital personnel from bodily injury and possible infection.

Vaccine and immune serum are available for prevention and control of rabies in humans. The vaccine currently available for administration is prepared from human fibroblasts and is called human diploid cell virus (HDCV). This is an inactivated vaccine that produces high levels of antibody. Immune serum globulin prepared from immunized human volunteers is also available in prevention. Both vaccine and immune serum are used in preexposure and postexposure prophylaxis. Preexposure prophylaxis consists of vaccination before exposure to the virus. Two intramuscular doses of HDCV are given after exposure, one each on days 0 (day of exposure) and 3. (Intradermal administration has been used to reduce the cost; however, antibody titers should be monitored because of less satisfactory antibody response in some cases.) For those individuals who do not receive preexposure prophylaxis, five doses of HDCV, one each on days 1, 3, 7, 14, and 28, and human rabies immune globulin (HRIG) on day 1, should be administered. The postexposure administration of HRIG is particularly important since vaccine alone cannot prevent rabies. Persons continually exposed to the risk of rabies should receive booster doses of vaccine at two-year intervals.

Immune serum may occasionally suppress the immune response of a vaccinated person. It is therefore important to monitor the antibody response in case additional boosters of vaccine are required.

Two methods have been successful in controlling rabies in domestic animal populations: vaccination of animals and removal of stray animals from the community. Several modified live virus and inactivated vaccines are available for immunization of dogs and cats. Transmission of rabies from dog to dog has been virtually eliminated. Most infections in dogs and other domestic animals now come from the bite of wild animals such as foxes, skunks, and raccoons.

Wildlife rabies control is difficult and relies on poisoning or trapping in areas where many rabid animals are in close contact with humans. These techniques have not been successful in controlling wildlife rabies. No rabies vaccine is available for wild animals. Individuals working with wild animals in zoos or exhibits are recommended to avail themselves of preexposure prophylaxis.

YELLOW FEVER

Yellow fever is an acute viral disease that is acquired by humans principally from the bite of the female *Aedes aegypti* mosquito. The virus belongs to the family Togaviridae, genus flavivirus. Since the members of this family are transmitted by arthropods, they are also called *arboviruses* (from *ar*thropod-*bo*rne virus). The yellow fever virus is a spherical RNA virus of cubic symmetry approximately 50 nm in diameter. The nucleocapsid is surrounded by a lipid layer and an outer layer of glycoprotein peplomers.

Pathogenesis

There is a 3- to 6-day incubation period, after the bite of an infected mosquito, before the appearance of symptoms. The initial symptoms include fever, jaundice, gastrointestinal symptoms, and vomiting of blood. The fifth and sixth days of the illness are critical because the patient will either begin to recover or succumb to the disease. Recovery is accompanied by permanent immunity.

Studies on nonhuman primates reveal that the yellow fever virus multiplies first in regional lymph nodes near the site of inoculation. The virus then

passes into the bloodstream and localizes in the liver, spleen, kidneys, and lymph glands. Death results from destruction of liver cells and extensive hemorrhage.

Epidemiology

Aëdes aegypti is the primary mosquito vector, but other species transmit the infectious agent in Africa. The mosquito acquires the virus during the viremic stage in the host and maintains virus in the salivary glands. Transovarial (maintained in sex cells) transmissions have been demonstrated in *A. aegypti* and may account for the survival of the virus during the dry season as well as epizootic phases. Yellow fever virus is also harbored by monkeys and is transmitted to humans by *Aedes* as well as *Haemogogus* species of mosquito. Disease transmitted to humans by mosquitoes that acquire virus from infected monkeys is called *jungle yellow fever*. Many species of treetop monkeys are susceptible to disease. Humans acquire the disease when they enter the jungle and are bitten by mosquitoes that have acquired virus from infected monkeys.

In 1981 and 1982, 368 cases of jungle yellow fever were reported from South American countries. Most cases were among males and those over 15 years of age. No urban cases of yellow fever have been documented in the Americas since 1943. Yellow fever has been virtually eliminated from North America. In areas endemic for yellow fever mortality may be as high as 40 percent.

Laboratory Diagnosis

Definitive diagnosis of yellow fever can be made only by isolating and identifying the virus. Presumptive diagnosis is made if a fourfold rise in titer between acute and convalescent sera is found. The complement fixation, hemagglutination inhibition, neutralization, or fluorescent antibody test can be used in the serological diagnosis.

Prevention and Treatment

There is no specific treatment for yellow fever. In severe cases, supportive care is required to prevent renal failure. Control of yellow fever depends on eliminating the mosquito carrier, *Aëdes aegypti* and other species. A live attenuated virus vaccine, 17D, induces an effective antibody level that persists for at least 10 years and is recommended for anyone traveling to areas where yellow fever is endemic. Since the vaccine is produced in chick embryo cell culture, it should not be administered to persons who are hypersensitive to eggs.

VIRAL ENCEPHALITIS

Encephalitis is an infection involving the brain and spinal cord. It may be caused by complications resulting from infection by a number of viruses (measles, mumps, and herpes simplex viruses), or it may be epidemic in nature and be caused by certain neurotropic viruses. The neurotropic viruses responsible for epidemics are arthropod-borne viruses or arboviruses. The major arboviruses in the United States are *western equine encephalitis (WEE) virus, eastern equine encephalitis (EEE) virus, St. Louis encephalitis (SLE) virus*, and *California encephalitis (CE) virus*.

Viruses causing WEE, EEE, and SLE are transmitted during the summer months during their enzootic cycle involving domestic birds and mosquitoes. Mosquitoes spread the virus from the enzootic cycle to clinical hosts such as humans and horses. EEE in horses is a severe disease resulting in death in up to 90 percent of the cases. In the United States 62 confirmed cases of arbovirus encephalitis were recorded in 1983. WEE was found primarily in North Dakota, South Dakota, and Minnesota; CE in Ohio, Illinois, Wisconsin, and Missouri; SLE in California and Arizona; and EEE in Massachusetts, New York, Florida, and Rhode Island. The CE virus is also transmitted to humans by the mosquito but is apparently harbored by rabbits, squirrels, and other small mammals.

Most cases of encephalitis are relatively mild and are characterized by fever, vomiting, headache, and stiffness of the neck. Severe cases progress from mild symptoms to coma and convulsions. The mortality in humans varies from as high as 60 to 70 percent in EEE to less than 3 percent in WEE.

Many who survive infection are left with paralysis, mental deterioration, or personality changes.

Diagnosis is made only by isolation of the virus. Suspensions of brain tissue from infected animals are inoculated into newly weaned mice or cell culture. Viruses are identified by hemagglutination inhibition with standard antisera.

The most effective method of control is through reduction or elimination of the mosquito vector. No vaccine has yet proved effective.

RHINOVIRUSES AND THE COMMON COLD

A number of viruses, both respiratory and nonrespiratory, produce symptoms that resemble the common cold. The rhinoviruses account for approximately 30 to 40 percent of these upper respiratory tract infections.

The rhinoviruses are a subgroup of the picornaviruses. There are about 89 serotypes of the rhinovirus subgroup, with biophysical and biochemical properties similar to those of the other picornaviruses. The rhinoviruses can be separated from the enteroviruses by determining their acid stability. Rhinoviruses lose their infectivity when they are subjected to pH's between 3 and 5, while enteroviruses are unaffected.

The highest incidence of rhinovirus-associated illness occurs in September and October. The incubation period for rhinovirus infections is 1–3 days. The most frequent symptoms of infection are coryza, sore throat, and cough. Fever is usually not present. In children rhinovirus infection may cause more severe respiratory disease such as bronchitis. Infection from one serotype provides protection from challenge with the same serotype for up to 2 years. Persons with low levels of type-specific antibody are asymptomatic but shed virus upon reinfection. Those with high antibody titers are immune to infection.

Diagnosis of the cold is made from observations of clinical symptoms. There are no procedures for direct examination of clinical specimens or for serological diagnosis. Rhinovirus can be isolated from clinical specimens. The latter are made into suspensions, then inoculated into tubes containing human fetal diploid cells. Cultures are observed daily for CPE, a rounding up of the cells.

Because of the mildness of colds and the great number of serotypes that may be involved, vaccine prophylaxis is not practical. Preliminary experiments with human volunteers, however, indicate that vaccines prepared from some serotypes are protective up to 6 months while others elicit no immunological response.

ENTEROVIRUSES

There are five groups of enteroviruses: the polioviruses, group A coxsackieviruses, group B coxsackieviruses, echoviruses, and enterovirus types 68 to 71. Polioviruses have already been discussed. Enteroviruses have been associated with respiratory disease, nonspecific fevers, and rash. In addition these agents may also cause CNS injury including paralysis, meningitis, and encephalitis. A few species have also been associated with myocarditis and pericarditis.

Most infections by the enteroviruses are asymptomatic, but certain individuals are more susceptible to disease than others. Nearly 60 percent of the cases of enteroviral disease are found in those under 10 years of age, while 25 percent of the cases are found in those under 1 year of age. Enteroviral agents are isolated primarily during the summer months, with the peak incidence in August. Polioviruses are usually isolated throughout the year.

Pathogenesis

Enteroviruses multiply in the alimentary tract before invading the bloodstream. The viremic stage is followed by passage of virus into the central nervous system.

Aseptic meningitis is caused primarily by echoviruses but coxsackieviruses A and B may also be involved. The symptoms of aseptic meningitis include headache, fever, and vomiting, followed by stiffness of the neck and muscular weakness. The disease is self-limiting.

Herpangina is an infection of the throat caused

primarily by group A coxsackieviruses. The disease is characterized by fever and sore throat with ulcerated lesions appearing on the mucous membranes of the oral cavity. Herpangina occurs primarily in children between the ages of 3 and 10 and is self-limiting.

Myocarditis is caused primarily by group A and B coxsackieviruses. Infection occurs in the newborn as well as in children and adults. Infection in neonates, which is often fatal, results from dissemination of the virus to a variety of organs. Rapidly developing cyanosis and circulatory collapse precede death. Most older children and adults recover from myocarditis, but permanent heart injury may occur.

Pleurodynia is a muscular disease caused primarily by group B coxsackieviruses. The illness occurs in children between the ages of 5 and 15 and their parents. Pain over the lower rib cage or upper abdomen is the most characteristic symptom. The illness is self-limiting, and most patients do not require bed confinement. The average length of illness is 4–7 days.

Treatment

There are no satisfactory antiviral agents for treatment of enterovirus disease (excluding poliomyelitis) other than providing supportive care, which includes bed rest. Hospital-associated outbreaks of disease, especially in neonates, necessitate isolation techniques to prevent transmission.

PARAINFLUENZA VIRUSES AND RESPIRATORY SYNCYTIAL VIRUS

The parainfluenza viruses and respiratory syncytial virus (RSV) belong to the family Paramyxoviridae, and both are associated with lower respiratory tract infection. Disease is classified as croup, tracheobronchitis, bronchiolitis, and pneumonia. All agents are more frequently isolated from young children. Infants younger than 4 months are usually spared from disease.

Parainfluenza Viruses

There are four parainfluenza antigenic types, but only types 1, 2, and 3 cause medically important infections. These three types account for nearly 40 percent of all isolates from lower respiratory disease. Types 1 and 2 cause epidemic outbreaks with peak occurrences in January and February, but type 3 is isolated throughout the year. Type 1 infections occur in the fall of years ending in an even number, while type 2 are observed in odd-numbered years. The reason for this is that infection by one virus interferes with the replication of the other virus type.

At birth maternally acquired antibody is sufficient to prevent disease in infants by types 1 and 2, but this is not true for type 3. Primary infections with types 1 to 3 usually cause coryza, pharyngitis, bronchitis, or a combination of these. The temperature of the infected is usually elevated to 100°F for 2–3 days. The most severe manifestation of disease is croup, which is characterized by a coarse cough and hoarseness. This can lead to fatal pneumonia. Most infections, however, do not require hospitalization. Types 1 and 2 are more frequently associated with croup, while type 3 causes croup as well as pneumonia and bronchiolitis.

Reinfections with parainfluenza viruses are common and may occur within 3 months or a year of primary infection. The symptoms of disease are less severe with reinfection.

Therapy for severe infections caused by parainfluenza viruses consists primarily of supportive care. No vaccine has yet proved to be effective in clinical trials.

Specific diagnosis of parainfluenza virus infection is made by recovery of the virus and identification by rapid methods such as immunofluorescence, which is as sensitive as tissue culture for identification.

Respiratory Syncytial Virus

Respiratory syncytial virus accounts for approximately 20 percent of the cases of lower respiratory disease in children. RSV infections occur yearly but with different patterns. January and February

are the months with the highest incidence of disease in even-numbered years; March and April, in odd-numbered years. Antibody passively transferred from mother to fetus is of protective value during the first 6 weeks after birth, but the antibody titer decreases by a factor of two each month thereafter. Thus children after 2 months are especially liable to infection with RSV. Infections are more severe in infants under 6 months of age than in older children and adults. RSV often causes bronchiolitis and pneumonia in infants. Infection does not confer a permanent immunity, and reinfections are common but also milder. In hospitalized infants the risk of infection is directly related to the length of hospitalization. The hospital staff plays an important role in transmission of virus. Through the handling of infants, staff members acquire infection, probably by self-inoculation with contaminated secretions carried on their hands or on fomites.

One study has shown that more than 50 percent of the children between the ages of 6 months and 4 years have no demonstrable antibody to RSV. Approximately 12,000 infants (0.4 percent) each year will require hospitalization for bronchiolitis and pneumonia associated with RSV infection. Many of them could be protected with an effective vaccine. Inactivated vaccines are not satisfactory. Indeed, administration of killed vaccine to infants results in a more serious lower respiratory tract infection, when the infant is later exposed to wild virus infection, than is experienced by unvaccinated infants. Some researchers suggest that RSV infection may in fact be an immunopathological reaction to the presence of serum neutralizing antibodies. Thus the neutralizing antibodies of maternal origin may be important in causing severe immunological responses when infants are exposed to virus. Trials are in progress for evaluation of attenuated vaccines. Investigators are also searching for temperature-sensitive mutants of RSV that would be restricted from growth in the lower respiratory tract but could grow in the cooler regions of the upper respiratory tract.

The antiviral agent ribavirin, a nucleoside analogue resembling guanosine, has been shown in clinical trials to be effective in lessening the severity of lower respiratory symptoms and diminishing the duration of viral shedding.

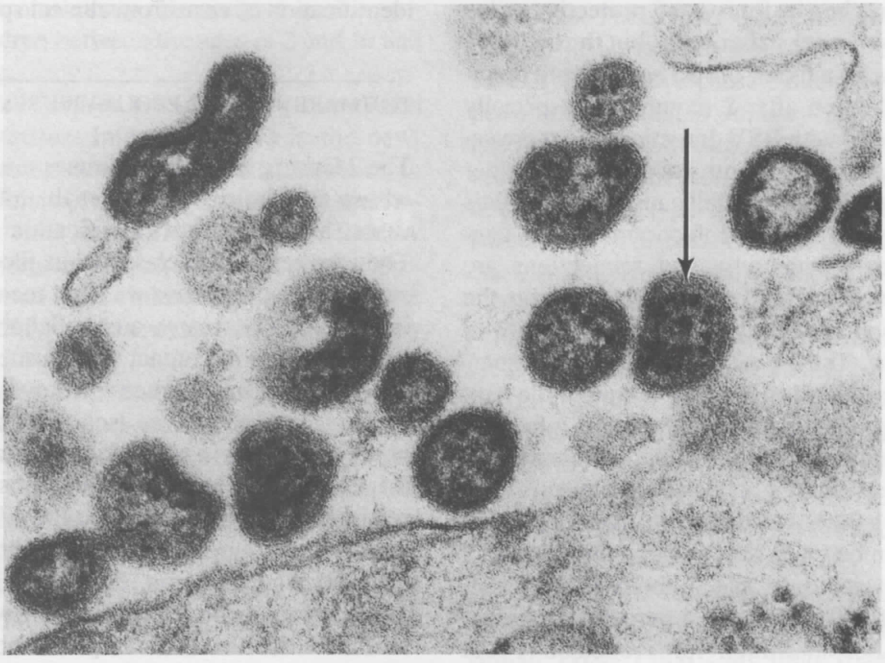
Immunofluorescence is the method of choice for identification of virus from clinical specimens.

THE MARBURG AND EBOLA VIRUSES

The Marburg and Ebola viruses are RNA viruses whose morphology resembles that of the rhabdoviruses, but a separate classification is recognized. They are tentatively classified as filoviridae. A disease of unknown cause was first recognized in 1967 in Marburg, Germany, among laboratory workers who had been in contact with tissues and cultures of imported African green monkeys. A virus called the *Marburg virus* was isolated. In 1976 several hundred persons in two African towns located near the Ebola River became ill, and most died. The virus isolated from them, called the *Ebola virus*, was morphologically similar but antigenically distinct from the Marburg virus. The life cycle of these two viruses remains undetermined. There is no conclusive evidence that African green monkeys are the natural reservoir for the Marburg virus, and the epidemiology of the Ebola virus is also unclear. From the initial index case human-to-human spread can occur. It appears to be associated with contact with blood or products contaminated with blood, such as needles and syringes.

Both the Marburg and Ebola viruses produce a similar clinical response. The incubation period is 3–8 days, and initial symptoms include fever, headache, and conjunctivitis. These are followed 2–3 days later by vomiting, leukopenia, and thrombocytopenia. Finally there is intravascular coagulation and gastrointestinal bleeding followed by shock and death. The case fatality rate may be as high as 100 percent unless supportive care is available to prevent intravascular coagulation. Diagnosis requires the isolation of virus from blood or oropharyngeal secretions and cultivation in cell culture. Inoculated cells are examined for viral antigen using immunological techniques.

Fig. 34-24. Electron micrograph (×90,000) of arenavirus (*arrow*) cultivated in Vero cells. Note the sand-like electron-dense particles in the virus. (Courtesy R. Graham and P Jahrling.)



THE ARENAVIRUSES

The arenaviruses (*arena* means "sand" in Latin) are a group of viruses that produce chronic infection in rodents. Some of these viruses can produce a highly fatal disease in humans. The members of this group include the *lymphocytic choriomeningitis virus*; the *Tacaribe group of viruses*, which includes the *Machupo* and *Junin* viruses associated with hemorrhagic fevers, and the *Lassa fever virus*.

The arenaviruses are spherically shaped single-stranded RNA viruses ranging from 80 to 130 nm in diameter The interior of the virus contains electron-dense particles that are believed to be host ribosomes (Fig. 34-24). Virus can be isolated from certain rodent species who shed virus in various excretions. Except for Lassa fever these diseases are not spread from person to person. A brief description of these agents is presented in Table 34-2.

ROTAVIRUSES

Rotavirus (*rota* means "wheel" in Latin) was first detected in humans in 1973 in electron micrographs of duodenal mucosa obtained from infants with *acute gastroenteritis* (Fig. 34-25). The rotavirus belongs to the family Reoviridae. The virus possesses a double-shelled capsid that encloses a double-stranded RNA composed of 11 segments. Rotaviruses have also been detected in the stools of various animals, including calves, piglets, foals, and lambs. Human and animal rotavirus strains are antigenically related and share a common group antigen; some share subgroup antigens.

Rotaviruses are considered one of the major causes of gastroenteritis in children younger than 2 years of age in both developed and developing countries. In temperate climates infections occur primarily in the cooler months, but in tropical cli-

Table 34-2. Arenaviruses Causing Hemorrhagic Fevers

Viral agent	Source of virus in nature	Mechanism of spread	Characteristics of human disease
Lymphocytic choriomeningitis virus	House mice or laboratory Syrian hamsters; found in the United States	Contact with mice, hamsters, or their excreta	Outbreaks among laboratory personnel working with Syrian hamsters; fever headache, but may become meningeal; seldom fatal
Junin virus	Wild rodents; found in Argentina	Contact with rodent-contaminated maize by field workers	See Lassa fever
Machupo virus	Mice; found in Bolivia	Contact with household environment contaminated with mice excreta	See Lassa fever
Lassa fever virus	Mice; found in West Africa	Contact with mice or their excreta in fields; human-to-human spread by direct contact, contact with diagnostic specimens, or parenteral inoculation of infected body fluids	Incubation period of 1–2 weeks; high fever, headache, and diarrhea; later, rash develops on face and neck; mouth lesions and respiratory symptoms; capillary leakage with shock followed by renal failure and moderate hemorrhage from gastrointestinal tract and genitourinary tract; shock syndrome leads to death; fatalities may be as high as 10–20 percent

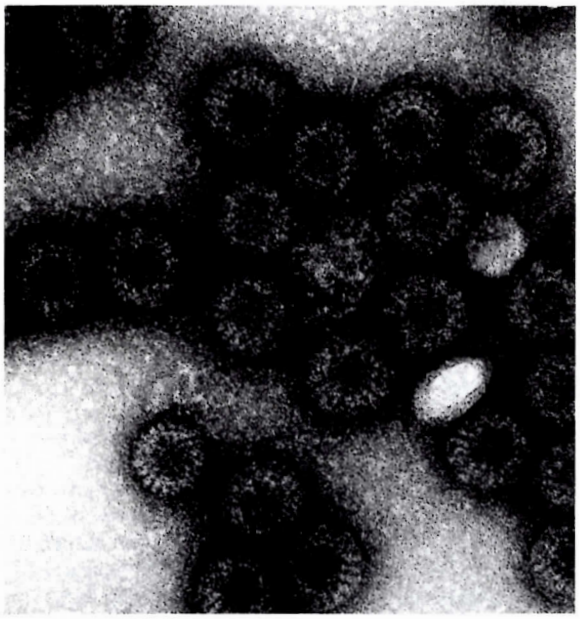
mates infections occur throughout the year The virus is believed to be transmitted by the fecal-oral route although it may be transmitted by food or water Most infections occur between 6 months and 2 years of age. Infection seldom occurs after 6 years of age.

The incubation period is approximately 48 hours. Fever, vomiting, and diarrhea are present at the onset of symptoms. The diarrhea and vomiting often become chronic, and the consequences are serious if no supportive therapy is given. In developing countries diarrhea (including diarrhea caused by other microbial agents such as *Shigella* and *Giardia*) can account for up to 20 percent of infant mortalities. The major factor causing death is dehydration and electrolyte imbalance.

Rotaviruses have only recently been cultivated on tissue culture. Identification and accurate diagnosis require visualization of particles by immunoelectron microscopy. Recently immunoassays have been refined, and monoclonal antibodies have been used in ELISA tests for rapid rotavirus identification.

The development of a vaccine is now a high priority, especially since it is now possible to produce enough rotavirus antigen in cell culture. One strategy that is being attempted is to use a calf rotavirus to immunize humans since the human and

Fig. 34-25. Electron micrograph of rotavirus. (Courtesy of H. Gelderblom.)



calf strain have similar group antigens. Calves inoculated in utero with bovine rotavirus were protected against challenge with human rotavirus.

Treatment of infection is directed at preventing dehydration and replenishment of water and electrolytes.

SLOW VIRUS DISEASES

Slow virus diseases represent a type of persistent infection (see Chapter 33). They are characterized by a chronic course that affects the central nervous system and are invariably fatal. Slow virus diseases are caused by conventional viruses that include measles virus (*subacute sclerosing* ***panencephalitis,*** or ***SSPE***) and polyomavirus (*progressive multifocal leukoencephalopathy*). The unconventional agents cause the diseases *kuru* and *Creutzfeldt-Jakob disease* in humans. Conventional agents can replicate in vitro, induce cytopathic effects, and initiate antibody formation in the infected host. The unconventional agents have not been isolated and cannot be demonstrated by electron microscopy. In addition, they do not induce antibody formation in the infected host.

KURU

Kuru is a chronic degenerative disease caused by a viral agent that has not been observed microscopically but can be transmitted to experimental animals. The disease was originally discovered among the Fore people of New Guinea. The incidence of kuru has decreased dramatically because of the elimination of the practice of cannibalism and the eating of dead kinsmen. Transmission probably occurs by autoinoculation of infected tissue through skin cuts, nasal mucosae, or conjunctiva. Women and children who handled infected brain tissue, and were the main participants in these ceremonies, were the most likely to be infected. The latent period may be as long as 20 years. The clinical symptoms include cerebellar incoordination and a shivering tremor involving head, trunk, and legs. Later the patient begins to stammer and there is incoordinate eye movement. These features worsen until the patient cannot walk and is unable to swallow. The patient dies within 3–9 months of onset of symptoms. There is no treatment for kuru.

CREUTZFELDT-JAKOB DISEASE

Creutzfeldt-Jakob disease is a degenerative disease of the CNS affecting persons between 35 and 65 years of age. Most human cases are sporadic. The disease can be transmitted to nonhuman primates. The infected patient becomes uncoordinated and demented, and death occurs within 9–18 months of onset of symptoms. Accidental transmission of this disease has occurred, although infrequently, during transplantation procedures, which suggests that the infectious agent is not limited to the brain. Both

kuru and Creutzfeldt-Jakob disease have pathological features similar to those of certain degenerative diseases of animals, that is, scrapie of sheep and transmissible encephalopathy of minks. There is no treatment for Creutzfeldt-Jakob disease.

Creutzfeldt-Jakob disease has recently been demonstrated to be caused by an infectious agent called a *prion*, which stands for *proteinaceous infectious particle*. Prions do not appear to be composed of any nucleic acid, yet they reproduce in a living cell. Investigations are currently underway to determine the method of reproduction.

SUBACUTE SCLEROSING PANENCEPHALITIS

Persistent infection of the central nervous system with measles virus is recognized as the cause of SSPE. The disease occurs most often in children and appears with a frequency of one case per million but in the Southeast this ratio is 4 to 5 cases per million. The majority of patients with SSPE had measles at an early age, most before 2 years of age.

SSPE follows natural measles infection after a latent period of about 7 years. The virus apparently remains in the nervous tissue in a suppressed form and then spreads through the brain without the formation of mature viral particles. SSPE-infected cells can be observed to have nucleocapsids in the nucleus and cytoplasm, but none are aligned at the cytoplasmic membrane for budding. SSPE patients have high titers of measles antibody in the cerebrospinal fluid, a characteristic not associated with natural measles infection. Apparently some nerve cells in the CSF die and release viral antigens that stimulate antibody production. The suppression of virus formation in nerve cells is believed to be due to the absence of a virus-specific protein or presence of a suppressive factor. The immune system of the host is unable to neutralize the virus because most virus is hidden intracellularly. The precipitation of disease 7 years after measles infection is believed to be due to some hormonal factor associated with puberty, but this is only a hypothesis.

The clinical findings of SSPE can be divided into 4 stages: Stage I, cerebral changes; Stage II, convulsive motor signs; Stage III, coma and spasms of the head, back, and lower limbs; Stage IV, loss of cerebral-cortical function and inability to speak. Most patients live an average of 18 months after diagnosis of SSPE.

Treatment of SSPE is still in an experimental stage. Some reports indicate that administration of transfer factor (see Chapter 17) can prevent the progression of symptoms. The drug isoprinosine has been reported to produce long-term remissions. The administration of measles vaccine in the United States has reduced SSPE greatly, but this is not true for developing countries where measles is still a major cause of morbidity and mortality in children.

PROGRESSIVE MULTIFOCAL LEUKOENCEPHALOPATHY

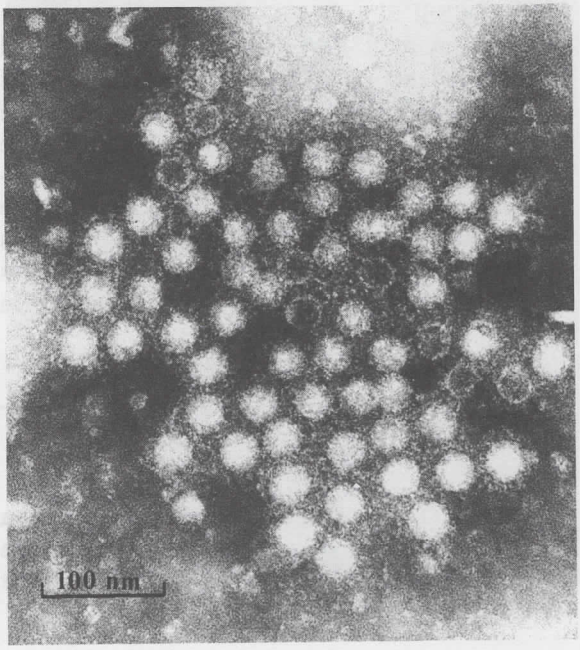
Progressive multifocal leukoencephalopathy is a rare demyelinating disease associated with the polyoma viruses, JC virus, and BK virus. These were discussed in Chapter 33.

THE HEPATITIS VIRUSES

The hepatitis viruses have only recently been classified, and in most textbooks they had previously been described as unclassified viruses. Although the hepatitis viruses include both DNA and RNA types they are still included together because of their similar pathogenicity.

Sporadic hepatitis can result from infection by several viruses, including cytomegalovirus, rubella virus, herpes simplex virus, and yellow fever virus. The principal agents of acute viral hepatitis are designated *hepatitis A virus* (*HAV*) *hepatitis B virus* (*HBV*), and *non-A, non-B hepatitis virus* (*NANB*). The infection caused by HAV is also called *infectious, epidemic,* or *short-term hepatitis*, while infection caused by HVB is termed *serum, transfusion*, or *long-incubation hepatitis*. The worldwide

Fig. 34-26. Immunoelectron microscopy of hepatitis A virus. (From A. MacGregor, et al., *J. Clin. Microbiol.* 18:1237, 1983.)



impact of these viruses is tremendous. Several hundred thousand people each year die from the late manifestations of hepatitis.

GENERAL CHARACTERISTICS

Hepatitis A Virus

Direct visualization of a virus-like particle associated with hepatitis A infection was not reported until 1973. The particles were prepared from stool specimens and observed by *immunoelectron microscopy* (*IEM*) (Fig. 34-26). In IEM the virus particles are coated with specific antibody from convalescent serum. This treatment produces large antigen-antibody aggregates that can be visualized.

HAV is a nonenveloped icosahedral virus that is approximately 28 nm in diameter. The viral genome consists of a single-stranded RNA molecule. The virus had not been appreciably characterized until 1982. At that time it was recommended that the agent be classified as enterovirus 72 but retain its original name, hepatitis A virus. When isolated from clinical material the virus must be passaged several times in marmosets before it can be used to infect cell cultures, and obtaining large quantities of the virus is difficult.

Hepatitis B Virus

HBV is a double-shelled virion 40 to 50 nm in diameter (originally called the Dane particle because of its discovery in 1970 by Dane) with a core 27 nm in diameter containing a double-stranded DNA molecule plus DNA polymerase. Tubular or filamentous forms up to 200 nm in length are also observed; they have a diameter of 22 nm. A third particle form, which is characteristically observed in the blood during persistent infection, is 22 nm in diameter (Fig. 34-27). These particles are composed of the same material as the surface antigen of HBV, which is called *hepatitis B surface antigen* (*HBsAg*) (originally called *Australia antigen*). A recently discovered antigen called the delta antigen (δ antigen) is found only in those with HBsAg. In patient serum the delta antigen is capsulated by HBsAg in 35- to 37-nm particles.

A new virus group has now been formed to include the HBV and three similar viruses that are found in woodchucks, certain ground squirrels, and Pekin ducks. The name proposed for this group is *hepadnavirus group*.

The surface antigen (HBsAg) manifests a group-specific determinant, *a*, and subtype-specific determinants *d* or *y* and *w* or *r*. With few exceptions, subtypes *d* and *y* have not been found simultaneously in the same patient, and subtypes *w* and *r* are also mutually exclusive. Of the four possible antigenic combinations—*adw, ayw, adr*, and *ayr*—the first three are epidemiologically predominant.

During the course of hepatitis B infection, several antigens and antibodies will appear. The following is a suggested system of nomenclature:

Hepatitis B virus—HBV, a 40- to 50-nm particle with a 27-nm core.

Hepatitis B surface antigen—HBsAg, found on the

Fig. 34-27. Electron micrograph (×210,000) of hepatitis B virus (HBV). Three morphological forms are observed: spheres 22 ± 2 nm in diameter, filamentous forms approximately 22 nm in diameter and varying in length, and 42-nm particles. (Courtesy G. A. Cabral and M. Patterson.)

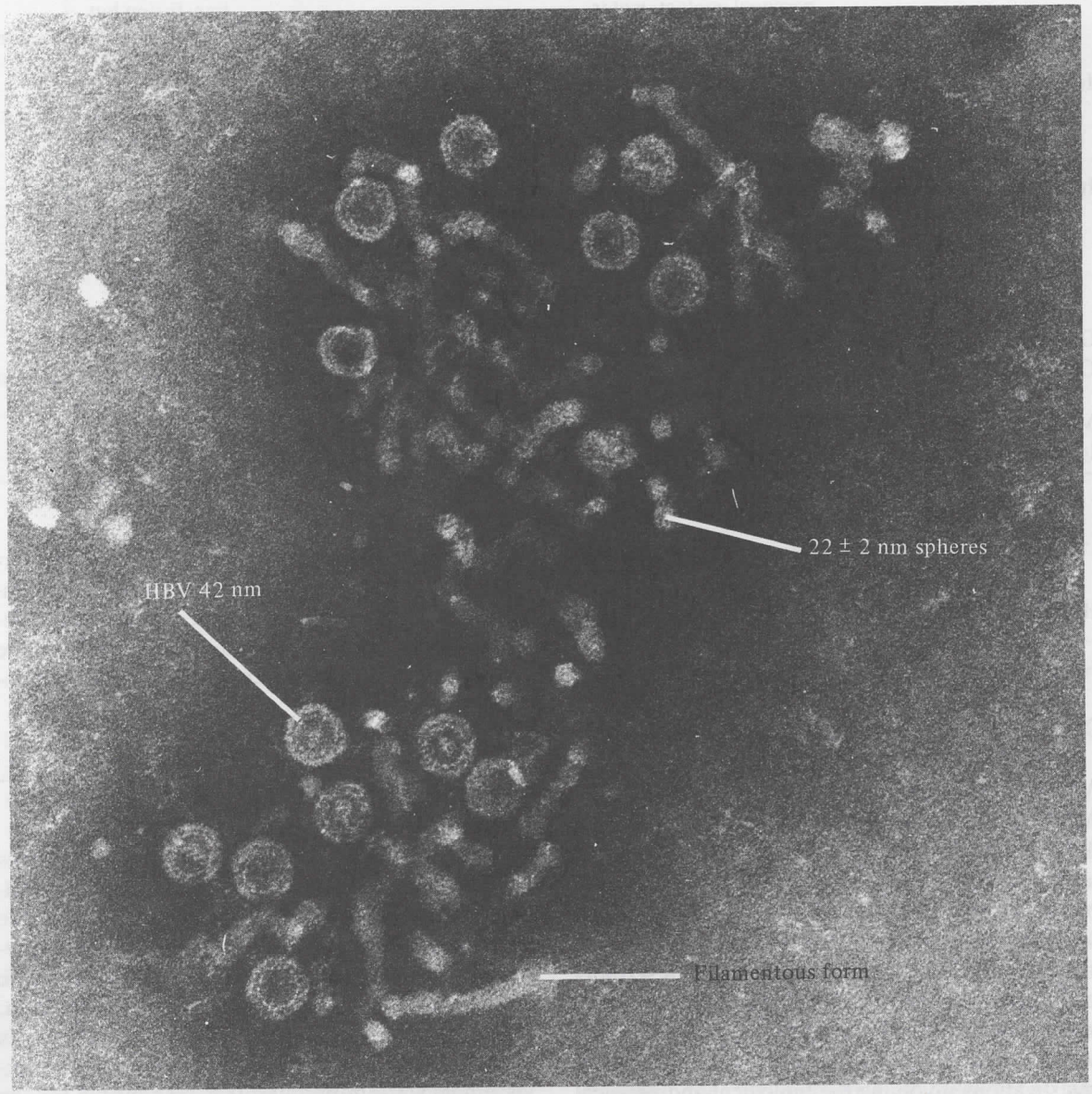


Table 34-3. Epidemiological and Clinical Features of Hepatitis Virus Infection

Characteristic	Hepatitis A	Hepatitis B	Non-A, Non-B
Transmission	Fecal-oral route (close personal contact; water and food as vehicles)	Parenteral, sexual contact	Primarily by blood transfusion but waterborne transmission possible
Incubation period	15–50 days	30–150 days	50–70 days for posttransfusion type but 10–40 days for waterborne type
Immunity to reinfection	Solid immunity to homologous agent but not to heterologous hepatitis agents	Reinfection with homologous agent possible but seldom occurs; no immunity to heterologous hepatitis agents	Reinfection believed to occur
Onset	Abrupt	Insidious	Insidious
Complications	Usually none	10% or more can become chronic	Chronic disease is common
Mortality	Less than 0.1%	1%	Similar to HBV
IgM presence	Persistently high levels	Only slightly elevated levels	?

surface of the HBV, tubular forms, and the unattached 22-nm particles.

Hepatitis B core antigen—HBcAg, found within the core of HBV.

Hepatitis Be antigen—HBeAg, closely associated with nucleocapsid of HBV.

Delta antigen—δ antigen, associated with HBsAg, as a 35- to 37-nm particle.

Antibody to HBsAg—anti-HBs

Antibody to HBcAg—anti-HBc

Antibody to HBeAg—anti-HBe

Non-A, Non-B Virus

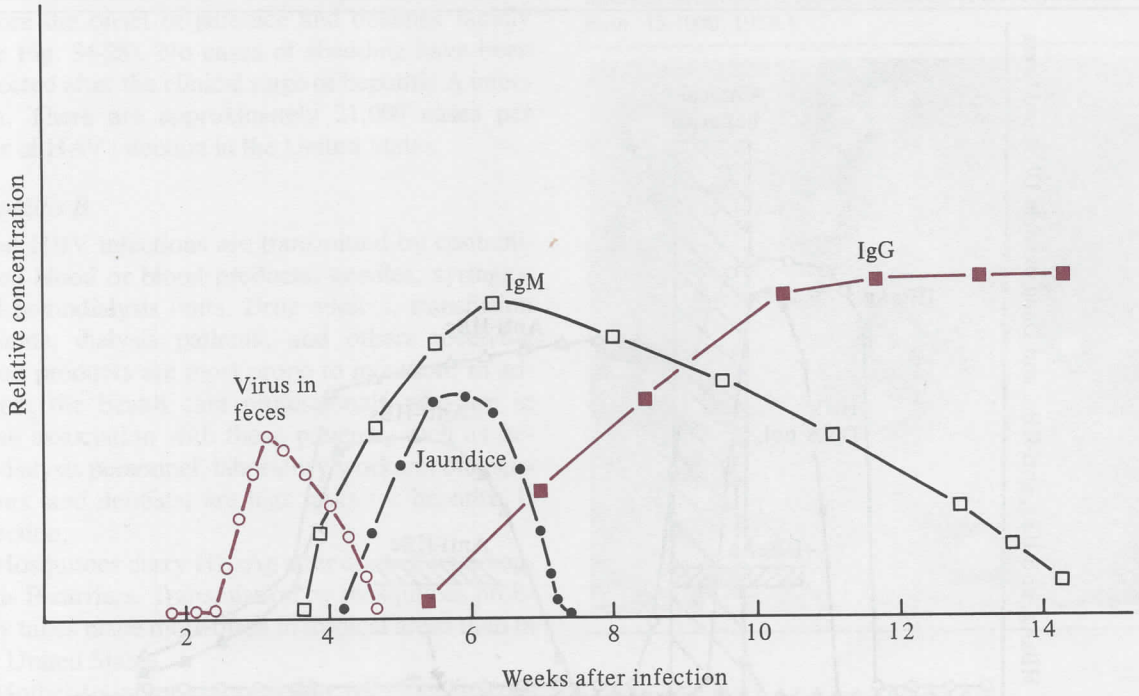
Serologically negative tests for HAV and HBV led to the discovery of a hepatitis caused by a serologically and morphologically unknown virus. The disease can be transmitted to chimpanzees and is now referred to as *non-A, non-B hepatitis*. Recent investigations involving the biological and physical characteristics of the hepatitis virus indicate that two, or possibly more, agents may be involved. Little is known of the physical characteristics of the viruses, but one of the suspected NANB agents is chloroform sensitive and is associated with the formation of cytoplasmic tubules and convoluted endoplasmic reticulum in infected liver cells (hepatocytes). Both suspected NANB agents are associated with ultrastructural changes in hepatocytes.

PATHOGENESIS

It is impossible to differentiate the clinical features of HAV, HBV, and NANB virus infection. Hepatitis viruses have an affinity for hepatocytes. In the case of HBV infection core components are apparently synthesized in liver cell nuclei, whereas the surface antigens are produced in the cytoplasm. The coat antigen is produced in great excess and is sometimes passed into the bloodstream as an unassembled 22-nm particle. It is responsible for the antigenemia associated with hepatitis B infection.

The incubation period of HAV infection is 15–40 days; of HBV infection, 30–150 days (Table 34-3). The first clinical symptoms of hepatitis virus infec-

Fig. 34-28. Events associated with hepatitis A virus infection. (○○○ = virus in feces; □□□ = IgM antibody in serum; ●●● = jaundice; ■■■ = IgG antibody in serum.) (From W. S. Robinson. In G. L. Mandell, R. G. Douglas, Jr., and J. E. Bennett [Eds.], *Principles and Practices of Infectious Diseases*. New York: Wiley, 1979.)



tion are usually fever, headache, and vomiting. The illness may be associated with jaundice (icteric) or there may be no jaundice (anicteric). Hepatic lesions caused by the hepatitis viruses are the same: parenchymal cell degeneration, necrosis, proliferation of reticuloendothelial cells, and inflammation. During the period of necrosis there is elevated serum amino transferase activity and bilirubin. Massive hepatic necrosis can lead to encephalopathy and death, but fortunately most patients recover from hepatitis B infections. HAV infection is much less severe than HBV infection and is associated with a self-limiting illness. HBV infections can lead to persistent infections and a severe outcome (a mortality rate as high as 10 percent). Persistence is not associated with HAV infections. The immunological and biological events associated with infections by HAV and HBV are outlined in Figures 34-28 and 34-29, respectively.

A direct association between hepatitis B viral infection and hepatocellular carcinoma has been surmised for many years but only recently detailed. In western Europe and the United States, where infection with HBV is relatively uncommon, primary hepatocellular carcinoma is a rare tumor (approximately 6000 cases per year). In Africa and Asia, where HBV is endemic, hepatoma is one of the most widely recognized carcinomas. In these endemic areas the risk for primary hepatocellular carcinoma in the carrier group is 245 cases per 100,000 population. When all hepatitis B markers are included 90 percent of hepatoma patients have evidence of present or past infection with HBV. The latent period from the time of HBV infection to ap-

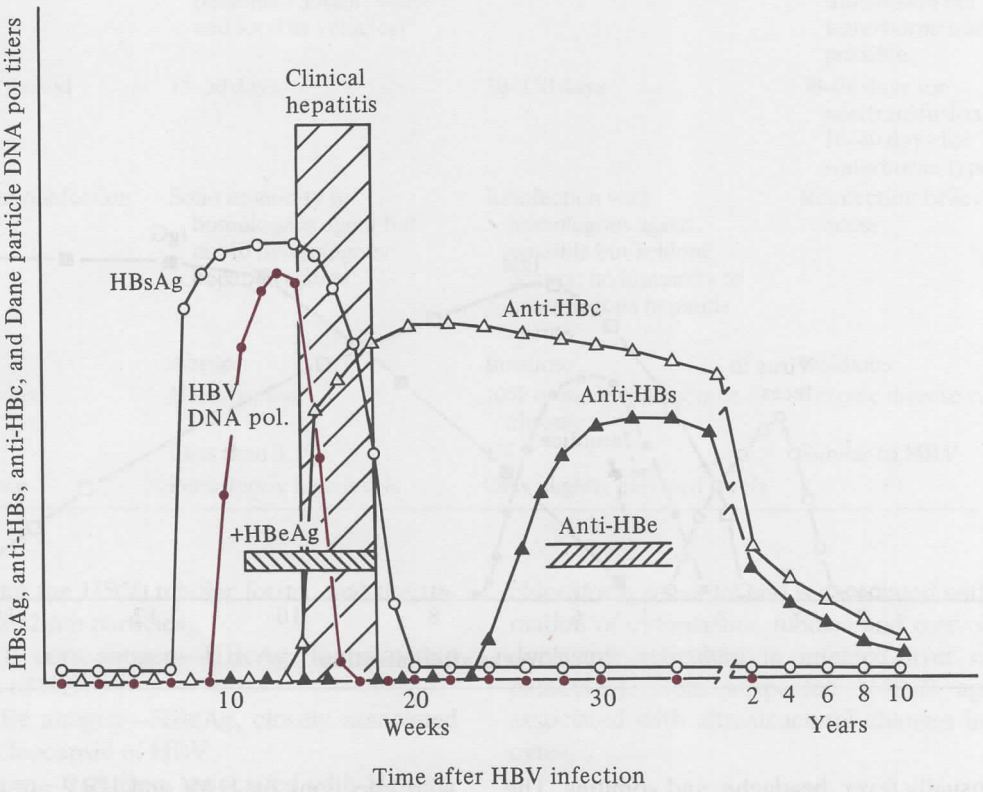


Fig. 34-29. Hepatitis B viral markers in the blood during the course of self-limited HBsAg positive infection. (HBsAg = hepatitis B surface antigen; anti-HBs = antibody to HBsAg; anti-HBc = antibody to hepatitis B core antigen; DNA pol = DNA polymerase.) (From W. S. Robinson. In G. L. Mandell, R. G. Douglas, Jr., and J. E. Bennett [Eds.], *Principles and Practices of Infectious Diseases*. New York: Wiley, 1979.)

pearance of the cancer is approximately 30 years. With nucleic acid hybridization techniques integrated HBV genome can be identified in most biopsy specimens of tumor tissue.

EPIDEMIOLOGY

Hepatitis A

Hepatitis A is usually transmitted by the fecal-oral route. Food, water, and milk, fecally contaminated, are potential sources of infection. Improperly cooked shellfish obtained from waters contaminated with raw sewage have also been implicated in transmission. Many outbreaks of infection have been traced to food handlers who, being actively ill, have contaminated products such as potato salad, doughnuts, and frozen custard (see boxed essay, p. 764). Hepatitis A may also be spread by contaminated blood and blood products, just as hepatitis B is; however, this is not the usual method of transmission. The highest incidence of infection occurs in children and adolescents. In developing countries where hygiene and sanitation may be poor, HAV infection occurs at an early age and 90 percent of the population have antibody to the virus. In developed countries only 20 percent of the population have antibodies to the virus. Sporadic outbreaks in the United States are frequently asso-

ciated with day-care centers and are related to those children less than 2 years of age in whom infection is less symptomatic. Virus is readily transferred to adult contacts. Shedding of virus occurs before the onset of jaundice and declines rapidly (see Fig. 34-28). No cases of shedding have been detected after the clinical stage of hepatitis A infection. There are approximately 21,000 cases per year of HAV infection in the United States.

Hepatitis B

Most HBV infections are transmitted by contaminated blood or blood products, needles, syringes, and hemodialysis units. Drug addicts, transfusion patients, dialysis patients, and others receiving blood products are most prone to infection. In addition, the health care professionals who are in close association with these patients, such as hemodialysis personnel, laboratory workers, oral surgeons, and dentists, are high risks for hepatitis B infection.

Mosquitoes carry HBsAg after contact with hepatitis B carriers. Transmission by mosquitoes probably takes place more often in tropical areas than in the United States.

Mother-to-infant transmission resulting in congenital hepatitis can occur in the following ways: transplacentally, oral route at delivery, from maternal blood or stool, or mother-infant contact immediately after birth. The outcome of hepatitis infection in the newborn is influenced by the route of infection, dose of virus, and presence of maternal antibodies directed against HBsAg or HBcAg.

Infectious virus has been recovered from the semen and saliva; thus venereal contact can lead to infection. The prevalence of HBV infection among homosexual men ranges from 51 to 76 percent. This is to be compared with 4.4 percent for blood donors, 40 percent of spouses and household members of HBsAg carriers, and 55 to 67 percent of parenteral drug abusers. The risk of infection among homosexuals is related to the frequency of sexual contact. Passive anal-genital intercourse is most strongly associated with HBV infection, followed by oral-anal intercourse and rectal douching in preparation for intercourse. Traumatized rectal tissue may serve as the portal of entry of HBV from infected semen. Oral-oral contact or oral-genital contact are not related to HBV infection.

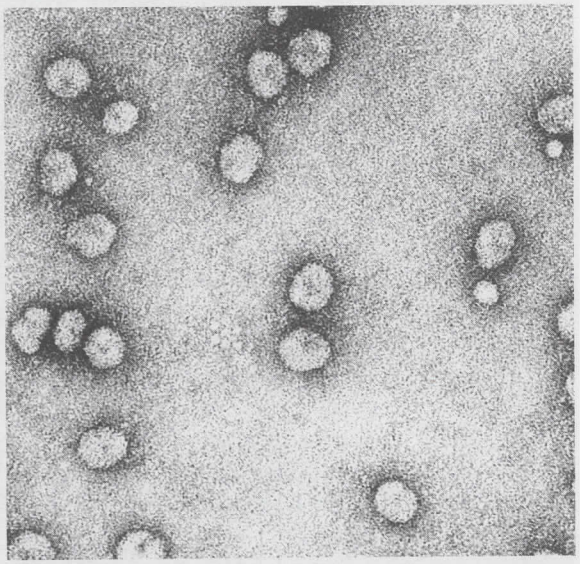


Fig. 34-30. Delta antigen. Electron micrograph ($\times$ 150,000) of spherical forms of delta-associated particles. They average 36 nm. Some are empty; others have internal structure. (From F. Bonino, et al., *Infect. Immun.* 43:1000, 1984.)

Patients who carry HBsAg for 20 weeks will usually become persistent or chronic carriers of HBV. There are believed to be 400,000 chronic carriers in the United States and 175 million chronic carriers worldwide. The most troubling aspect of these figures is that mortality related to infection is highest in the chronic carrier. Persistent carriers have high levels of HBsAg as well as anti-HBc in the serum. Chronic hepatitis can be divided into two types: chronic persistent and chronic active. Chronic persistent hepatitis is characterized by little damage to the liver, and there is no progression to severe liver damage. Chronic active hepatitis is characterized by chronic inflammation and necrosis of liver cells. The most important consequence of chronic active hepatitis is the progression to cirrhosis, which occurs in 50 percent of the affected individuals. The

OUTBREAK OF FOODBORNE HEPATITIS A—NEW JERSEY

An increase in the number of hepatitis cases in Monmouth County, New Jersey, was reported to the New Jersey Department of Health on June 15, 1981. Investigation by state and local area health departments revealed that 56 cases of hepatitis had occurred during the first 3 weeks of June in an area of Monmouth County where the usual average is 3 or 4 cases per month. Patients for whom appropriate laboratory tests had been done were confirmed to have hepatitis A.

Detailed food histories revealed that, within the appropriate incubation period for hepatitis A, 55 (98 percent) of the 56 patients had eaten at a Mexican-style restaurant. Interviews of a control group matched for age, sex, and neighborhood of residence, showed that 10 percent of the controls ate food from this restaurant over a comparable time period. The restaurant agreed to close voluntarily pending further investigation.

Of the patients whose illness was related to the Mexican restaurant, 71 percent were male, 68 percent were between the ages of 15 and 29 years, and four were children under 15 years. A case-control study using 46 nonill patrons revealed that patients were more likely to have eaten nachos, beans, and jalapeno peppers. Both beans and jalapeno peppers were used in preparing nachos.

Ten individuals including the two owners worked in the restaurant; all handled food at one time or another. Interviews on June 18 revealed that one employee who frequently ate food from the restaurant was ill with hepatitis at the time of the interview. Another employee had symptoms compatible with hepatitis on May 9. He had worked all day May 9, but felt too ill to work thereafter; the diagnosis of hepatitis A was confirmed for him on May 16. This employee prepared food—including grating cheese, shredding lettuce, and occasionally cutting meat—measured portions of meat, beans, jalapeno peppers, onions, cheeses, and lettuce into shells; and served the customers.

Because a food handler was recently ill with hepatitis and because the restaurant was implicated in the spread of hepatitis, immune globulin (IG) was offered to all individuals who ate in the restaurant from June 5 until it closed. A total of 1430 people were immunized at a 2-day clinic held June 19 and 20.

If acute hepatitis A has been confirmed in a food handler by testing for IgM-specific HAV antibody, immunoglobulin prophylaxis (IG, gamma globulin) may be considered for patrons, depending on the probability of transmission of infectious virus and the probability of successful intervention in transmission by using IG. However, few food handlers actually appear to transmit disease via food, and IG prophylaxis of patrons is seldom warranted. Although for the past few years approximately 1000 food handlers with non-B hepatitis have been reported annually to CDC, an average of four outbreaks of foodborne hepatitis A have been reported each year.

Most reported outbreaks have been traced to symptomatic rather than asymptomatic excreters. Uncooked foods have most frequently been associated with foodborne hepatitis because normal cooking temperatures inactivate HAV. However, cooked foods that were handled after cooling and foods that were contaminated and then cooked with insufficiently high internal temperature to inactivate HAV have also been implicated. Although poor hygiene practices among food handlers increase the chance of transmission of virus, outbreaks have occurred even when food handlers' personal hygiene practices were described as "acceptable" and "generally good." Hygiene practices should be assessed by interviewing the ill food handler, co-workers, and employer. If deficiencies occurred and the ill food handler did not wear gloves, prophylaxis may be considered for patrons who ate implicated food items during the appropriate time period. Successful intervention in disease transmission depends on identifying persons at risk and administering IG within 2 weeks after exposure.

Other employees who have been regularly exposed to the index case are at risk of acquiring infection. If they do become infected, they may serve as additional sources of infection for future food consumers. These employees should be extremely conscientious in their hygiene practices, and those who handle high-risk foods should be given IG. Screening of co-workers for elevated liver enzymes or antibodies to HAV does not appear justified because the enzymes are not specific for hepatitis A, and both enzymes and antibodies appear after most virus excretion has occurred.

From *MMWR* 31:150, 1982.

bility of viral persistence are age of patient at time of primary infection, immune status at time of infection, sex of the patient, severity of the primary infection, size of the viral inoculum, and genetic factors. Neonatal infection is associated with the highest rates of persistence. The rate decreases during childhood, but it is still considerably higher than in adults. Studies of those receiving hemodialysis indicate that altered immune responses predispose the patient to persistent infection. The more severe the primary infection the less likelihood there is for the development of persistence. The more concentrated the viral inoculum the less likely a person will become a carrier. Finally males are more likely to become persistent carriers than females; the male-to-female ratio is greater than 2 to 1.

Delta antigen or delta virus, first discovered in Italy in 1977, is found only in those who are HBsAg carriers (Fig. 34-30). The delta virus is defective and requires HBV to be synthesized. Anti–delta antigen (antibody to delta antigen) has been found worldwide, but its prevalence is high only in chronic HBV carriers in Italy. Anti–delta antigen is low in most countries except for drug users and polytransfused hemophiliacs.

Non-A, Non-B Hepatitis

NANB virus is responsible for 90 to 95 percent of the cases of post-transfusion hepatitis. The source of transfused blood influences the incidence of infection by NANB agents. NANB hepatitis is found in 6 to 7 percent of recipients of blood from volunteer donors, compared with 17 to 35 percent of those receiving blood from paid donors. Twenty-five to 50 percent of the sporadic cases of NANB

hepatitis have been shown to be unrelated to blood transfusions. A waterborne epidemic of viral hepatitis in Kashmir, India, involved over 275 patients and was believed to be associated with NANB agents.

LABORATORY DIAGNOSIS

HAV and anti-HAV can be detected by immunoelectron microscopy, but this technique is impractical for routine diagnosis. Radioimmunoassay (RIA), immunofluorescence, and ELISA will all detect HAV; RIA and ELISA will also detect antibody (anti-HAV). The presence of non-class-specific anti-HAV has limited diagnostic value because anti-HAV IgG persists for life. Anti-HAV IgM, however, is almost always present at onset of disease and usually becomes undetectable within 4 to 6 months. Detection of anti-HAV IgM is a powerful tool for recognition of current or recent hepatitis A.

Immunofluorescence or immunoperoxidase staining and electron micrograph can be used to identify HBV, but they are not applicable to routine screening. Identification procedures today involve a variety of serological tests for detecting antigen or antibody. These tests include agarose gel diffusion, counterelectrophoresis, rheophoresis, complement fixation, hemagglutination, immune adherence, ELISA, and RIA. A variety of commercial kits that eliminate the need to prepare viral antigen or antibody are now available. Much research has been directed at finding specific methods for detecting hepatitis infection as well as differentiating chronic persistent from chronic active carriers of hepatitis. This differentiation is important since chronic active cases often require steroid therapy, which has its own risks. The research has revealed the following serum components that may help in hepatitis diagnosis:

1. Anti-HBs. Antibody to HBsAg is present during convalescence and is associated with immunity to reinfection with HBV. Elevated levels of anti-HBs beyond convalescence may indicate a persistent infection.
2. Anti-HBc. Antibody to HBcAg is present during convalescence. It is associated with HBV replication and persistence in chronically infected individuals.
3. Anti-HBe. Antibody to HBeAg is infrequently observed in chronic infections. Patients with anti-HBe are usually not infectious, usually have normal liver histology, and have a good prognosis.
4. HBeAg. HBeAg is considered a marker for infectivity and may be characteristic of acute or chronic disease.
5. HBcAg. The quantitative amount of HBcAg and HBsAg components in liver tissue, together with histological descriptions of liver cells, may be useful in determining the prognosis of infection. Low HBcAg or none in patients seropositive for HBsAg indicates persistent infection with little liver damage. A high level of HBcAg is associated with marked liver damage and doubtful prognosis.

IMMUNITY

Hepatitis A

During the course of disease antibodies are produced against various hepatitis antigens. For HAV infections IgM antibodies appear about 3 weeks after infection and about 1 week before the appearance of symptoms, and reach a maximum during the time that clinical symptoms have reached their peak (see Fig. 34-28). IgG antibodies become most evident 10–16 weeks after infection, that is, they are part of the convalescent sera (see Fig. 34-28). Antibodies to HAV can be detected in the serum many years after infection. HAV infection produces a solid immunity and reinfections do not occur; however, infection by HBV or NANB virus is possible.

Hepatitis B

From 4 to 8 weeks after infection HBsAg appears in the blood, yet antibodies to the surface antigen do not make their appearance until many months after the illness has terminated (see Fig. 34-29). An-

tibodies to the core antigen (anti-HBc) are produced and can be detected during the clinical manifestations of disease. They decline with a decline in anti-HBs but can be detected 5–6 years after infection. Acute hepatitis B infection is usually of the *a* type specificity, which appears to confer resistance to reinfection by the same or different subtypes. Anti-HBs confers immunity to reinfections, but it has been observed that even when anti-HBs titers are undetectable resistance to reinfections may still occur. This implies that cell-mediated immune responses are probably involved in resistance.

Non-A, Non-B Hepatitis

Infection by NANB does not appear to confer a solid immunity. Studies with various animals have produced inconclusive results.

PREVENTION AND TREATMENT

No therapeutic measures have proved to be of any benefit during the course of acute hepatitis. Some drugs are under clinical investigation for treatment of persistent infection; these include interferon and vidarabine (ara-A) and ara-AMP.

Hepatitis A Prevention

Fecal contamination of food, water, milk, and eating utensils is a frequent cause of hepatitis A epidemics. Hepatitis A infections can be prevented by improving sanitation conditions and through the rapid detection of carriers. Careful hand washing after contact with a person with HAV or contact with contaminated objects is important in preventing infections. Food handlers should be screened for hepatitis infection before they are hired. Human gamma globulin affords some protection if given during the incubation period of the disease by reducing the severity of infection. Gamma globulin is recommended in preventing the spread of infection in crowded institutions or among people in high-risk occupations (military personnel, missionaries) and those traveling to areas where hepatitis A infection is endemic.

Hepatitis B Prevention

Blood and blood products must be carefully screened for hepatitis B antigen (HBAg) to eliminate the risk of infection. To prevent post-transfusion hepatitis, blood donors must also be screened before their blood is collected. A great reduction in post-transfusion hepatitis has resulted from the use of volunteer blood donors. The use of dialysis units at home rather than in the hospital for known HBsAg carriers has also substantially reduced cross infections.

HBAg deposited on various surfaces is unusually resistant to chemical and physical agents and thus serves as a potential source of environmental contamination. Cross infection using dialysis equipment is very common since the monitoring devices contain connector elements that fit inside disposable blood tubing side arms. Connectors should be disposable, or they should be disinfected with sodium hypochlorite (0.5%). Dentists, oral surgeons, hospital personnel who work with dialysis equipment, drug addicts, and people who are likely to come in contact with blood or blood products should wear gloves whenever possible. The equipment and instruments (e.g., syringes, needles) used in practice should be either disposable or sterilized to prevent infection.

Treatment of blood and blood products is difficult because of the unusual resistance of the virus. Sodium hypochlorite is virucidal when used on equipment containing minimal amounts of proteinaceous material. For whole serum an equal volume of full-strength sodium hypochlorite is sufficient to inactivate HBsAg.

The hepatitis virus heated for 1 minute at 90°C will lose its infectivity but not its antigenicity. Thus serum containing inactivated virus used as a prophylaxis before infection with HBV can reduce the severity of hepatitis B infection.

Studies of the effect of passive immunization are encouraging. Hepatitis immune globulin (HBIG) made from plasma pools containing high titers of anti-HBs is recommended for postexposure prophylaxis of individuals sustaining needle punctures or mucosal exposure to blood known to contain HBsAg, family members of acute or chronic car-

Table 34-4. Characteristics of Diseases Caused by DNA Viruses

Viral disease	Viral agent	Epidemiology	Clinical manifestations	Vaccine
Smallpox	Poxvirus	Person to person via direct contact; begins as respiratory infection	Lesions in internal organs and eruptions on skin	No longer available to general public; disease eradicated
Herpesvirus diseases				
Cytomegalovirus disease	Cytomegalovirus	Direct contact	Congenital disease can result in fetal abnormalities; opportunistic for transplant patients (jaundice, hepatosplenomegaly); mononucleosis-like disease from transfusions	Experimental vaccine for renal transplant patients
Chickenpox	Varicella-zoster virus	Direct contact with respiratory secretions	Viremia followed by rash; usually self-limiting	Yes*; immune globulins also available
Shingles	Varicella-zoster virus	Activation of latent varicella virus	Skin lesions similar to those of chickenpox but more painful	No
Cutaneous herpes	Herpes simplex virus 1	Direct contact; activation of endogenous virus	Lesions on face	No
	Herpes simplex virus 2	Activation of endogenous virus but can be transferred sexually	Lesions on genitalia	No
Infectious mononucleosis	Epstein-Barr virus	Direct contact with respiratory secretions	Fatigue, chills, fever, spleen and liver enlargement	No
Respiratory and ocular disease	Adenoviruses	Direct contact	Bronchitis, croup, pneumonia, conjunctivitis	Yes for military recruits
Epidemic acute gastroenteritis	Norwalk agent	Fecal-oral	Diarrhea	No
Long-term hepatitis	Hepatitis B virus	Contact with contaminated blood or blood products; sexual transmission also possible	Jaundice, usually self-limiting, but chronic disease very serious	Yes; immunoglobulins also available

*Vaccine has not yet been licensed in the United States.

riers, and infants of chronically infected mothers. HBIG is also given in conjunction with hepatitis B vaccine to health workers following exposure to blood containing HBsAG and to homosexual men following exposure to an HBsAg-positive man. Preexposure prophylaxis is advised for patients and staff in dialysis units, oral surgeons, physicians, dentists, drug addicts, family members of acute and chronic carriers, and infants of chronically infected mothers. If HBIG is not available, standard immune serum globulin (ISG) containing some anti-HBs may confer protection. Great care should be taken in determining who should be immunized because HBIG is expensive and ISG has given equivocal results. Under certain circumstances immunization is not required. Persons who carry antibodies to HBeAg and lack DNA polymerase probably will not require HBIG. The level of DNA polymerase is a measure of the effectiveness of HBV synthesis in the patient. During pregnancy it is important to determine whether seroconversion from HBeAg to anti-HBe has taken place. Mothers with circulating HBeAg give birth to babies who eventually acquire HBsAg, whereas those with anti-HBe give birth to infants without hepatitis. Immunization for post-transfusion hepatitis is of little value since most cases are caused by a non-A, non-B agent.

A hepatitis B vaccine licensed in 1981 was made available to the general public in 1982. The vaccine is a suspension of inactivated 22-nm surface antigen particles purified from human plasma. Primary adult vaccination consists of three intramuscular doses, the second and third given at 1 and 6 months, respectively, after the first. The duration of protection and need for boosters has as yet not been determined, but vaccine does produce protective antibody in more than 90 percent of healthy individuals, including infants. Vaccination is recommended only for certain high-risk groups and not for the general population. Preexposure vaccination is recommended for health care workers, hospital staff, clients and staff of institutions for the mentally retarded, hemodialysis patients, homosexually active men, users of illicit injectable drugs, recipients of certain blood products, household and sexual contacts of HBV carriers, and inmates of long-term correctional facilities. Postexposure vaccination is recommended for infants born to mothers who are chronic carriers. Vaccination of the infants is recommended at 3 months of age. Vaccination may also be appropriate for sexual and household contacts of patients with acute hepatitis B and health care workers who receive a needle stick from HBsAg-positive patients.

Non-A, Non-B Hepatitis Prevention

There is no vaccine for NANB hepatitis. Blood donors with elevated levels of serum alanine aminotransferase are more likely to transmit NANB hepatitis to blood recipients than are donors with normal levels of the enzyme. Testing of serum for enzyme levels may permit exclusion of high-risk donors.

SUMMARY

Because of the length of this chapter the summary consists of Tables 34-4 and 34-5, which describe the characteristics of the viral diseases.

Table 34-5. Characteristics of Diseases Caused by RNA Viruses

Viral disease	Viral agent	Epidemiology	Clinical manifestations	Vaccine
Measles	Measles virus	Direct contact with respiratory secretions	Fever and rash associated with viremia; usually self-limiting; subacute sclerosing panencephalitis a rare sequela of measles	Yes; immune globulin also available
Mumps	Mumps virus	Direct contact with respiratory secretions	Salivary glands enlarged after viremia; usually self-limiting	Yes
Rubella	Rubella virus	Congenital rubella during viremia in mother; postnatal infection via respiratory secretions	Congenital disease can result in death of fetus or deformities at birth; rash in postnatal disease and usually self-limiting	Yes; immune globulin also available
Influenza	Influenza viruses A, B, and C	Contact with respiratory secretions	Cold-like syndrome but in compromised patients can be complicated by pneumonia	Yes
Poliomyelitis	Poliovirus	Fecal-oral	Fever and stiffness of neck; usually self-limiting; 1% develop paralytic disease	Yes
Rabies	Rabies virus	Bite of infected animal or inhalation of contaminated aerosols	Fever, difficulty in swallowing, convulsive seizures, coma, and almost invariably death	Yes for animals and humans; immune globulin also available
Yellow fever	Yellow fever virus	Bite of infected mosquito (*Aedes aegypti*)	Jaundice, extensive liver damage	Yes; immune globulin also available
Encephalitis	Arboviruses (western equine, eastern equine, California, and St. Louis encephalitis virus)	Bite of mosquito	Fever, headache, stiffness of neck; fatalities high in eastern equine encephalitis	No
Colds	Rhinoviruses	Contact with respiratory secretions or contaminated fomites	Coryza, sore throat, and cough	No

Table 34-5 (continued)

Viral disease	Viral agent	Epidemiology	Clinical manifestations	Vaccine
Enterovirus disease	Polioviruses, coxsackieviruses A and B, echoviruses, and enterovirus types 68–71	Direct contact with respiratory secretions	Aseptic meningitis, herpangina, myocarditis, and pleurodynia	No
Lower respiratory tract disease	Parainfluenza viruses 1, 2, and 3; respiratory syncytial virus	Direct contact with respiratory secretions	Croup, bronchiolitis, pneumonia	No
Hemorrhagic fevers	Marburg virus, Ebola virus	Contact with blood (?)	Fever, headache, intravascular coagulation, and shock precede death	No
	Arenaviruses (lymphocytic choriomeningitis Tacaribe group, and Lassa fever viruses)	Contact with rat secretions	High fever, headache, diarrhea, capillary leakage, and shock	No
Short-term hepatitis	Hepatitis A virus	Fecal-oral	Jaundice; recovery permanent and no persistence	Yes
Epidemic gastroenteritis	Rotavirus	Fecal-oral	Diarrhea accompanied by vomiting and fever	No

QUESTIONS FOR STUDY

1. Since smallpox has been eradicated who now receives the vaccine?
2. What antiviral drugs are specifically used for infections of the eye?
3. What viruses are considered latent and can be reactivated to cause disease?
4. What viruses are transmitted congenitally and cause fetal harm?
5. What viral infection is common in the renal transplant patient?
6. What are the causes of the following conditions or syndromes: subacute sclerosing panencephalitis, Reye's syndrome, and zoster?
7. What is the concern about vaccines in the prevention of diseases caused by latent viruses?
8. What types of clinical syndromes are associated with herpes simplex type 1? Type 2?
9. What drugs are being used in the treatment of genital herpes?
10. What age group is most severely affected by the Epstein-Barr virus?
11. What viruses are associated with the common cold? What other clinical manifestations are these viruses capable of inducing?
12. What virus is responsible for venereal warts?

13. What viruses are associated with diarrheal disease? How are they isolated?
14. What are some of the severe affects of mumps virus infection in the adult?
15. Who should be vaccinated with the rubella vaccine? Why is this vaccination so important?
16. What is the distinction between antigenic drift and antigenic shift as regards the influenza viruses?
17. How has the improvement of hygienic practices caused a change in the epidemiology of polioviruses?
18. What are the advantages and disadvantages of the inactivated and live polio vaccines?
19. What are Negri bodies?
20. What are the principal reservoirs of rabies virus in nature?
21. What is the distinction between jungle and urban yellow fever?
22. What are arboviruses?
23. What viruses are responsible for primary aseptic meningitis?
24. What virus accounts for 20 percent of the cases of lower respiratory disease in children?
25. What is the distinction in the epidemiology of hepatitis A, B, and NANB viruses?
26. What hepatitis B antibodies are present at high levels in chronic disease?
27. For whom is preexposure hepatitis B vaccine needed?
28. What is the significance of anti-HBe in the serum of hepatitis B victims?

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VII. MEDICAL MYCOLOGY

35. THE PATHOGENIC FUNGI

OBJECTIVES

To understand the phenomenon of dimorphism and its relationship to fungal disease

To differentiate between sexual and asexual reproduction

To differentiate among systemic, subcutaneous, cutaneous, superficial, and opportunistic mycoses on the basis of how the causal agent enters the body, and the tissues and organs affected

To describe how fungal pathogens are detected in the laboratory

To describe the most important opportunistic fungal pathogens and the conditions that lead to their association with disease

To describe the important mycotoxins and how humans become intoxicated

- Characteristics of the Fungi
  - Morphology
  - Growth and Reproduction
    - Yeasts
    - Molds
  - Pathogenesis
  - Laboratory Diagnosis
  - Immunity
  - Treatment and Prevention
  - Importance to Humans
  - Classification
- Fungal Diseases
  - Systemic Mycoses
    - Histoplasmosis
      - Epidemiology
      - Pathogenesis
      - Laboratory Diagnosis
      - Treatment
    - Coccidioidomycosis
      - Epidemiology
      - Pathogenesis
      - Laboratory Diagnosis
      - Treatment
    - Blastomycosis
      - Epidemiology
      - Pathogenesis
      - Laboratory Diagnosis
      - Treatment
    - Paracoccidioidomycosis
      - Epidemiology
      - Pathogenesis
      - Laboratory Diagnosis
      - Treatment
    - Cryptococcosis
      - Pathogenesis
      - Laboratory Diagnosis
      - Treatment
  - Subcutaneous Mycoses
    - Sporotrichosis and Other Subcutaneous Mycoses
      - Pathogenesis
      - Laboratory Diagnosis
      - Treatment
  - Dermatophytoses
    - General Characteristics
    - Laboratory Diagnosis
    - Treatment
    - Tinea Capitis (Ringworm of the Scalp)
    - Tinea Corporis (Ringworm of the Body)
    - Tinea Pedis (Ringworm of the Feet)
    - Tinea Barbae (Ringworm of the Beard Area)
    - Tinea Favosa (Ringworm of the Scalp)
    - Tinea Unguium (Ringworm of the Nails)
    - Tinea Imbricata (Ringworm of the Torso)
    - Dermatophytes and Allergy
  - Superficial Mycoses
    - Tinea Versicolor
    - Tinea Nigra Palmaris
    - Black and White Piedra
  - Diseases Caused by Opportunistic Fungal Pathogens
    - Candidiasis
      - General Characteristics
      - Pathogenesis
        - Cutaneous Candidiasis
        - Systemic Candidiasis
        - Chronic Mucocutaneous Candidiasis
      - Laboratory Diagnosis
      - Treatment
    - Aspergillosis
      - Pathogenesis
      - Laboratory Diagnosis
      - Treatment
    - Mucormycosis
      - Pathogenesis
      - Laboratory Diagnosis
      - Treatment
- Mycotoxins (Mycetismus and Mycotoxicosis)
  - Mycetismus
  - Mycotoxicosis

The fungi are eukaryotic microorganisms found predominately in the soil and are infrequent causes of disease in a variety of animals including humans. The study of fungi is called *mycology*. The infrequency of fungal disease in humans does not detract from their importance in medicine. The mortality for some fungal diseases is especially high, partly because they resemble bacterial diseases and are misdiagnosed, and partly because most occur in compromised patients in whom the immune response is poor. A greater understanding of the morphological, physiological, and pathogenic characteristics of fungi is an important aspect of diagnostic microbiology.

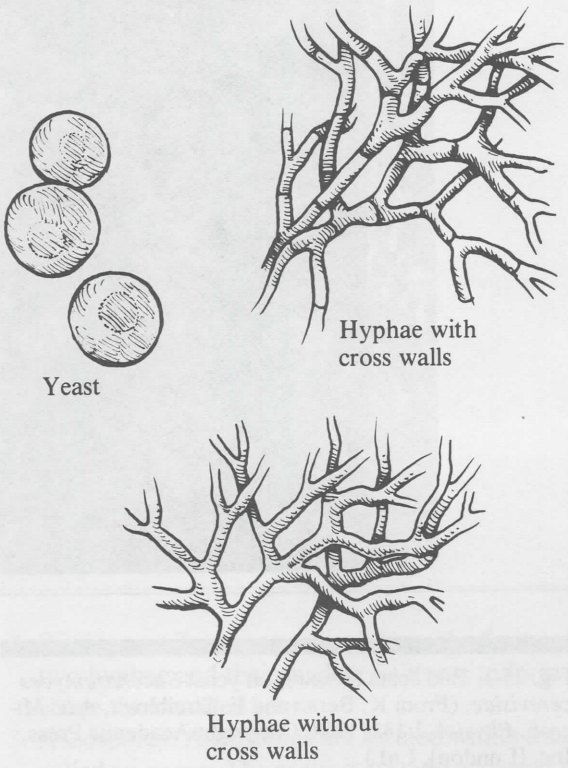
CHARACTERISTICS OF THE FUNGI

MORPHOLOGY

The fungi are divided into two morphological forms: *yeast* and *molds*. Yeasts are typically round or oval single cells that measure approximately 4 to 5 μm in diameter (Fig. 35-1), but some may be as large as 25 μm in diameter. Molds are multicellular fungi composed of filamentous or tubular structures called *hyphae* (sing. *hypha*). A single hypha may be from 5 to 50 μm in length and 2 to 4 μm in diameter. Some hyphae are partitioned by cross walls called *septa* (Fig. 35-1) that divide them into individual compartments. Each compartment contains cytoplasm and one or more nuclei. Not all hyphae are septate. In hyphae without septa the cytoplasm and nuclei circulate freely. Thus each hypha may contain several nuclei. These hyphae are referred to as *aseptate* and represent a mass of nucleated protoplasm. Hyphae grow by branching and apical extension and, when they do, form a mat of intertwined hyphae called a *mycelium*.

Not all fungi grow exclusively as yeasts or molds. Some fungi, which are called *dimorphic*, can exist in either state, depending on chemical and physical factors. Dimorphism plays an important role in the pathogenicity of certain fungi, as will be discussed later in the chapter.

Fig. 35-1. Characteristic morphology of yeasts and molds. The filamentous molds illustrated are depicted with cross walls called *septa* (sing. *septum*), but not all molds possess septa.



GROWTH AND REPRODUCTION

Yeasts

Yeasts may reproduce by sporulation, fission, or budding, but most reproduce by budding, that is, blastospore formation. During the process of budding a protuberance is formed on the mother cell surface (Fig. 35-2). As the protuberance enlarges there is a thickening of the cell wall at the neck connecting the mother cell to the bud. As the bud enlarges the neck or septum develops to the point where it separates mother cell from bud. The release of the bud leaves a permanent scar, called the *bud scar,* on the surface of the mother cell (Fig. 35-

Fig. 35-2. Scanning electron micrograph of yeast (*Y*) cells (*Candida albicans*) adhering to the mitral value (*MV*) of the heart. Some of the cells (*arrows*) are in the process of budding. (From S. A. Klotz et al., *Infect. Immun.* 42:374, 1983.)

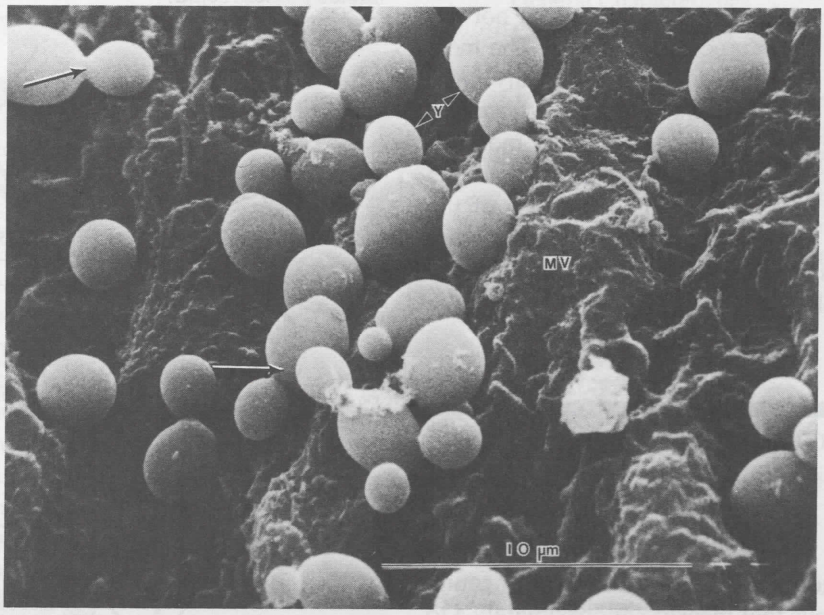
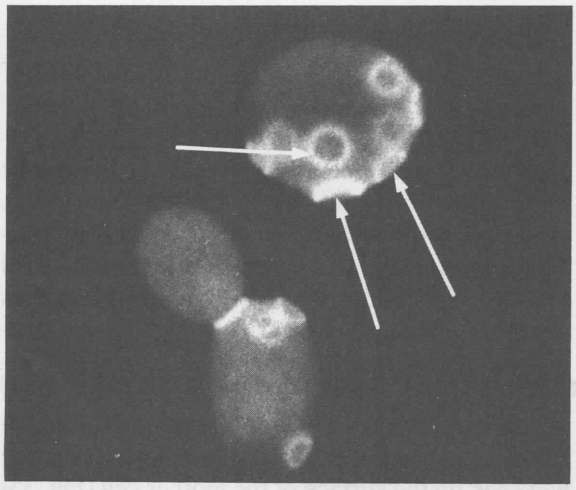


Fig. 35-3. Bud scars (*arrows*) on yeast *Saccharomyces cerevisiae*. (From K. Beran and E. Streiblova, *Adv. Microb. Physiol.* 2:143, 1968. Copyright Academic Press, Inc. [London], Ltd.)



3). Sometimes the developing bud also reproduces by budding. If this process continues without separation a chain of buds called *pseudohyphae* will appear (Fig. 35-4). Binary fission similar to the process that occurs in bacteria also occurs in yeast. Sporulation in yeasts may occur by sexual or asexual means and is similar to sporulation as it takes place in molds (see Molds). The yeasts pathogenic for humans are few. The one that causes most infections is *Candida albicans*. Other *Candida* species plus *Cryptococcus neoformans* and *Trichosporon cutaneum* are also pathogenic for humans but only rarely.

Molds

The mycelium of a mold may be divided into a vegetative portion, which remains attached to the substrate or penetrates it to obtain nutrients, and a reproductive portion, which is usually represented by aerial structures. The aerial structures can be ob-

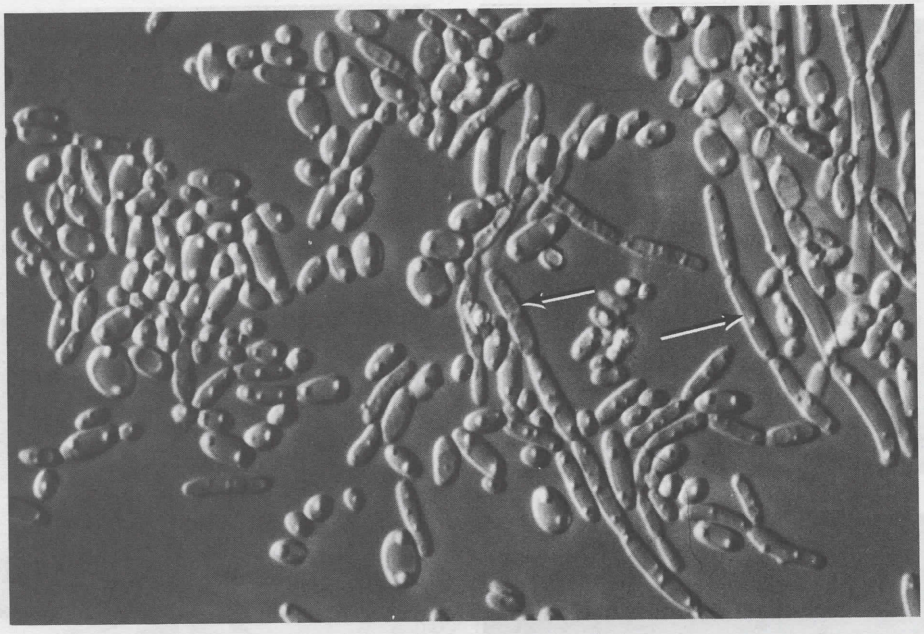


Fig. 35-4. Pseudohyphae formation (*arrows*) in *Candida*. (Nomarski interference microscopy, ×400.) (From M. Ogawa et al., *Appl. Environ. Microbiol.* 46:912, 1983.)

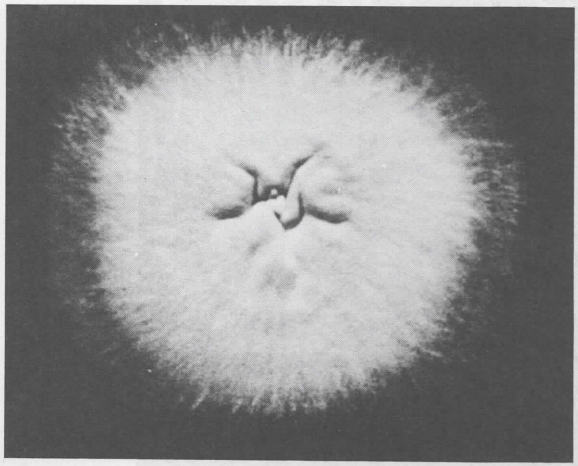
served in the laboratory as well as in nature as fuzzy or cottony filaments (Fig. 33-5).

Reproduction in molds is by formation of spores, which are produced through asexual or sexual processes. Sexual reproduction is accomplished by a fertilization process involving male and female sex cells (gametes). Sexual reproduction is an infrequent form of reproduction for most fungi in the laboratory or in infected humans, but it frequently occurs in nature, especially on plants. The sexual phase of several species has not as yet been detected, and this is the basis of classification for one group of fungi, that is, Fungi Imperfecti, Deuteromycetes (imperfect because they lack a perfect or sexual stage of reproduction). Sexual spores may be of various types:

1. Oospores. Oospores result from the fertilization of a specialized female structure called an *oogonium* by a male fungal structure called an *antheridium*.
2. Zygospore. Zygospores result from the fusion of two hyphae and their nuclear contents to form a single thick-walled spore.
3. Ascospores. Ascospores are formed within a sac called an *ascus*. The ascus is usually enclosed in a fruiting body called an *ascocarp*. Ascocarps vary in size and shape.
4. Basidiospores. Basidiospores are formed from the end of a club-shaped structure called a *basidium*. The fungi associated with such structures are mushrooms, puffballs, and other large fungi found in nature (Fig. 35-6).

Asexual spore formation is the most common type of reproduction among fungi (Fig. 35-7). Those fungi that form spores only asexually or not at all are referred to as being in the *imperfect state*. Until recently most human fungal pathogens were characterized as being in the imperfect state, but several species have been induced to produce spores by sexual means. Asexual spores may be

Fig. 35-5. A. Fungal colony that produces aerial structures. B. Mucoid colony that produces no aerial structures.



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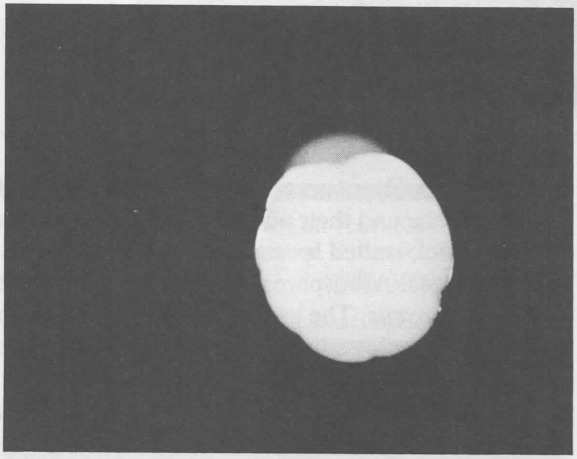


B

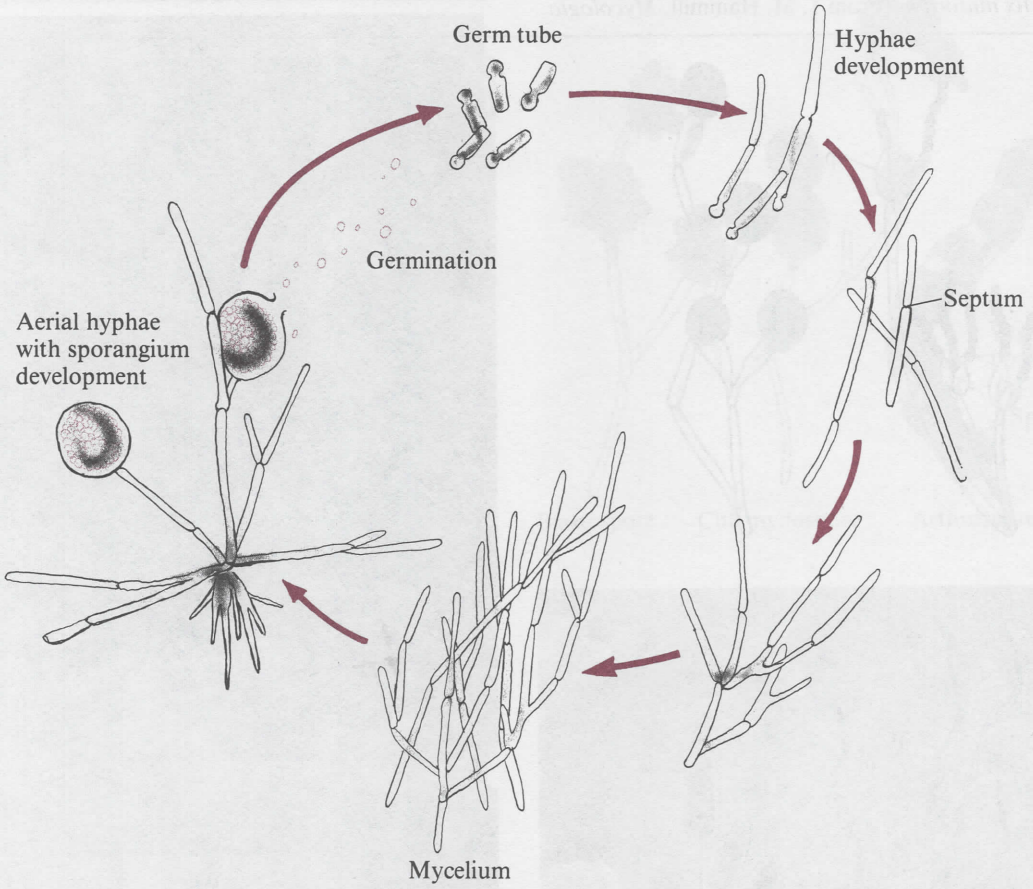
Fig. 35-6. Edible mushrooms. (Courtesy Carolina Biological Supply Co.)

produced in specialized structures that arise from hyphae, or they may be formed by modification of hyphae. *Conidia* are asexual spores produced on specialized stalks called *conidiophores* (Fig. 35-8) from which the spores are pinched off and released into the environment. Conidia vary in size, shape, and color (Fig. 35-8), a characteristic that lends itself to classification. Some asexual spores are produced within a specialized sac called a *sporangium* and are called *sporangiospores* (Fig. 35-9). Asexual spores arising directly from hyphae are collectively called *thallospores* and are divided into the following types:

1. *Arthrospores*. Arthrospores are barrel-shaped, thick-walled spores produced by the fragmentation of hyphae (Fig. 35-10).
2. *Chlamydospores*. Chlamydospores are sometimes referred to as *resting spores* because they are produced in older, dried-out cultures. They are produced by a swelling of the hyphal fragment and are thick walled (Fig. 35-10).
3. *Blastospores*. Blastospores represent a simple budding from the parent cell. They may appear as single daughter cells or as clustered cells, called *pseudophyphae*, that have not been detached (Fig. 35-10).

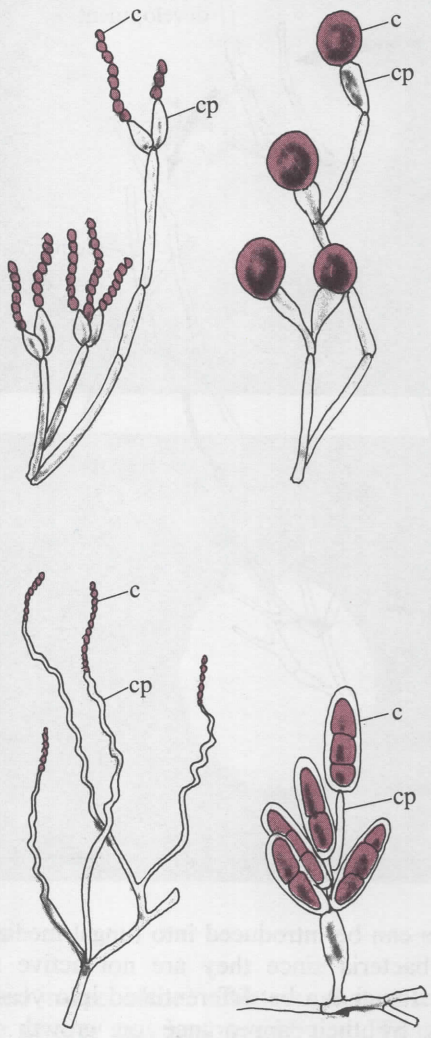
All molds are aerobic, while yeasts are facultative anaerobes and because of their biosynthetic

Fig. 35-7. Stages in the asexual production of a mold. Mycelium develops aerial sporangium in which spores are produced and later released into the environment. Spores germinate and develop germ tubes, which later multiply and form hyphae.



potential can grow on a variety of substrates that ordinarily would not support the growth of many bacteria. Fungi can tolerate pH's between 2.0 and 9.0, but abundant growth occurs between pH 5.0 and 6.0. In the laboratory fungi are cultivated on special media since they are recovered from specimens that are contaminated with bacteria. One of the most widely used isolation media for fungi is called *Sabouraud* agar, which contains maltose and peptone as its principal ingredients. It is selective against most bacteria because of its low pH. Antibiotics can be introduced into fungal media to inhibit bacteria since they are not active against fungi. Fungi can be differentiated into yeasts and molds by their appearance on growth media. Yeasts produce creamy opaque colonies, while mold colonies are fluffy and cottony (see Fig. 35-5). Dimorphic fungi when cultivated at 37°C appear as yeasts, but at 30°C they exist in a mold-like state. Differentiation of some fungal species can be made by demonstrating thermal dimorphism.

Fig. 35-8. Conidia. A. Diagram of different morphological forms of conidia (*c*) and the conidiophores (*cp*) that bear them. B. Photomicrographs of two types of conidia (*arrows*). Top, Phase-contrast photomicrograph of *Gliomastix murorum*. (From T. M. Hammill, *Mycologia* 73:229, 1981.) Bottom, Differential interference phase-contrast micrograph of *Cladosporium* species. (From M. R. McGinnis, *Laboratory Handbook of Medical Mycology*. New York: Academic, 1980.)



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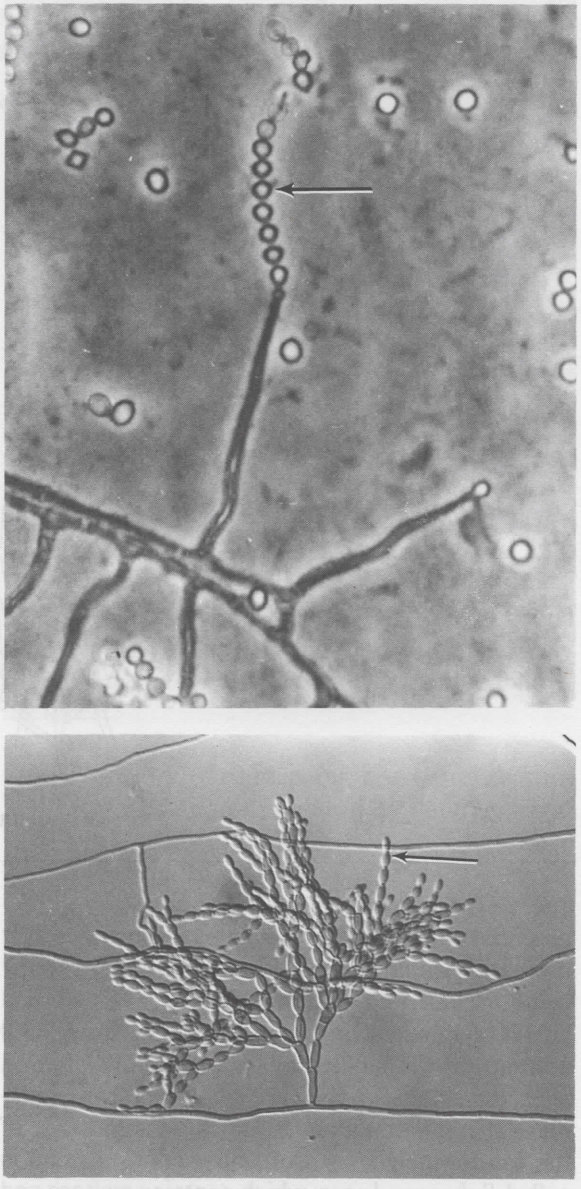


B

Fig. 35-9. Photomicrograph of a species of *Rhizopus* demonstrating the sporangium (*arrow*) and its encased sporangiospores (*SP*). (From E. J. Bottone, *J. Clin. Microbiol.* 9:530, 1979.)

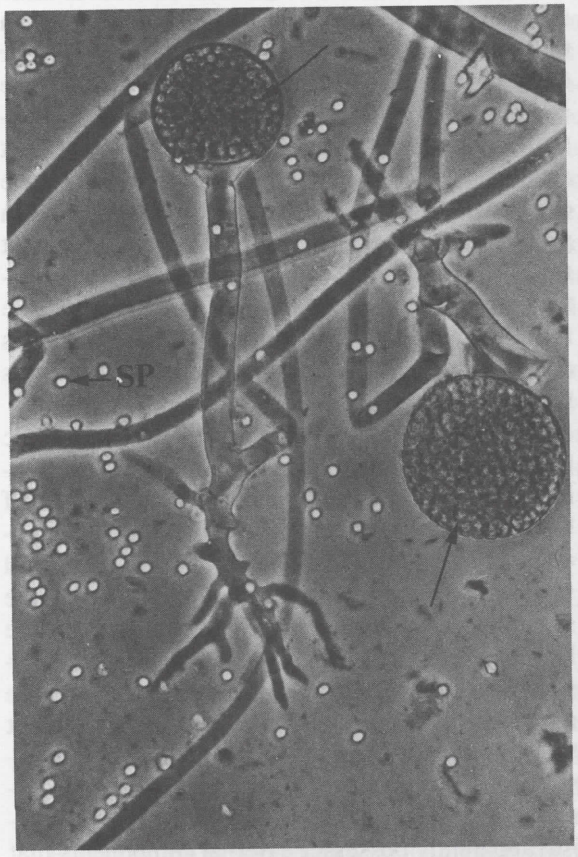
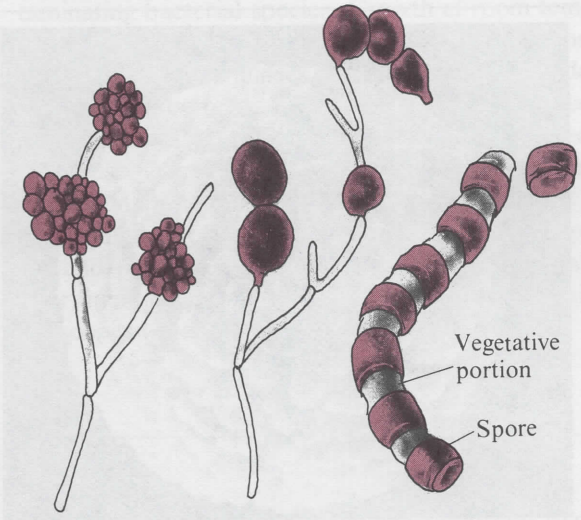


Fig. 35-10. Types of thallospores. A. Diagram. B. Scanning electron micrograph of arthrospores. (Courtesy D. J. Bibel.)



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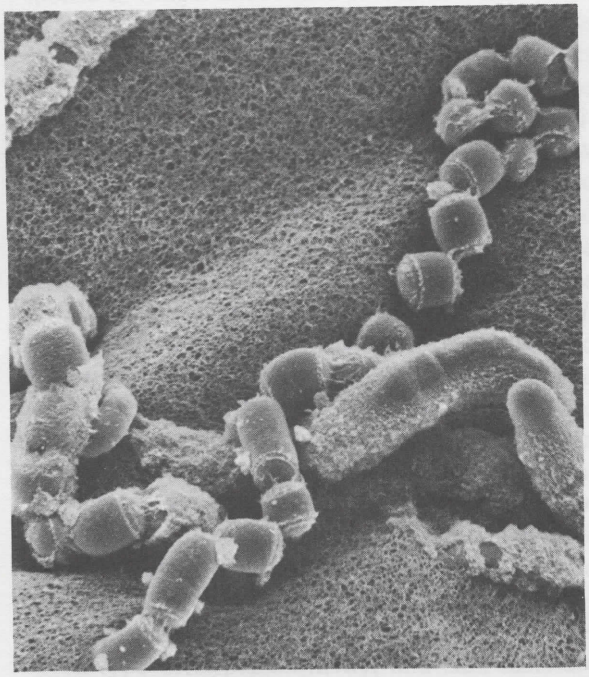
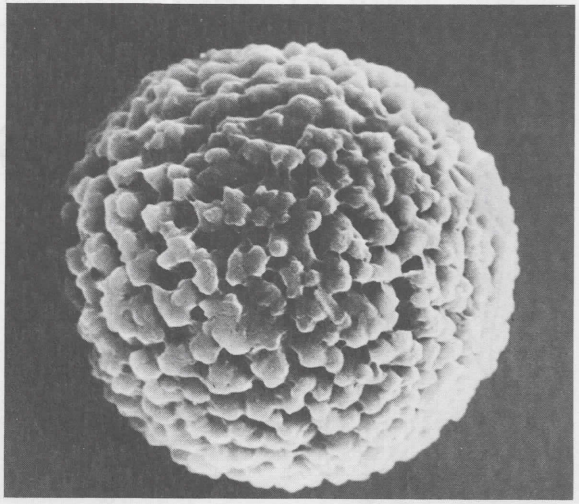


B

Fig. 35-11. Scanning electron micrograph (×2500 before 25% reduction) illustrating the surface characteristics of one type of fungal spore. (From J. S. Gardner et al., *Mycologia* 75:333, 1983.)



PATHOGENESIS

Fungi as we have already mentioned are found primarily in the soil where they degrade organic material. Hundreds of thousands of spores are released by fungi into the environment to be disseminated by air or water. Spores possess surface components that make them resistant to environmental factors (Fig. 35-11). The surface is usually a glycoprotein or glycoprotein-lipoprotein complex that is hydrophobic, thus permitting dissemination in the air. Spores are often inhaled or ingested by humans, yet fungal diseases are very rare in humans. Some spores are able to infect humans because of physical factors that force them into the host, for example, the lodging of spores in hair follicles. Some spores are present on sharp objects (wood, thorns) that can penetrate subcutaneous tissue. Inhaled spores are sorted out by the respiratory system based on size and density. Only under unusual circumstances do ingested spores cause disease. When the infectious fungal unit, which may be an arthrospore, conidium, blastospore, or other type, makes contact with host tissue, it must germinate and, depending on host response, may or may not initiate infection. The diseases caused by fungi are called *mycoses*. Most fungal infections in humans result from occupational hazards, for example, in those who are constantly in contact with the soil or those compromised by underlying disease or some condition that depresses the immune system. Laboratory workers who handle fungal cultures are at an increased risk of infection. Fungal spores are easily disseminated from a mold, but this is not a problem with yeast colonies. Mold cultures must therefore be handled with extreme care. Fungal infections can occur in the healthy adult only if the fungal infectious units are in excessive numbers and they make contact with a tissue that supports their growth.

Very little is known concerning the pathogenic mechanisms of fungi. None appear to produce toxins or toxic metabolites, which are characteristic of bacterial pathogens and which influence the outcome of infection. Most fungi that reach a mucosal surface are readily attacked by the host's immune system and are walled off. This response by the host prevents fungal reproduction and tissue invasion. Some fungi, when they make contact with the mucosal surface, induce an allergic response, but tissue invasion does not occur. Most of the research on the pathogenesis of fungi has been directed at the yeast *Candida albicans*. This organism apparently possesses external components that enable it to attach to mucosal surfaces. The attachment process occurs while the organism is in a yeast state, but invasion of tissue appears to require the formation of pseudohyphae. The appearance of pseudohyphae is believed to enable the fungus to escape phagocytosis, so the host must rely on other less efficient mechanisms of defense. This pleomorphic response is also characteristic of most fungi that cause systemic disease. Dimorphic fungi other than *Candida* are usually found in the yeast state in tissue, but outside the host they exist as filamentous forms. It appears that phagocytes are less efficient in killing yeast than they are the spore, which is the morphological form that is deposited

on the mucosal surface. In addition, some yeast-like cells are approximately 20 to 25 μm in diameter, a size that prohibits efficient digestion by immune cells of the host. Not all fungal pathogens that invade the host tissue exist in the yeast state; for example, *Aspergillus* species exist as hyphae in tissue (this organism's pathogenesis will be discussed more extensively later). Another mechanism for resisting immune defenses is found in the yeast *Cryptococcus neoformans*. This organism is inhaled as a spore and germinates in the host to form a yeast, which quickly produces a capsule. Encapsulation apparently permits the organism to resist phagocytosis. Other examples of apparent pathogenic mechanisms will be discussed later with the individual organisms.

Fungal diseases can be broken down into four types based on the tissue infected: *systemic, subcutaneous, cutaneous*, and *superficial*. In addition a separate group, diseases caused by opportunistic fungal agents, will be discussed.

LABORATORY DIAGNOSIS

The manifestations of many fungal diseases are very similar to those of diseases caused by bacterial agents such as *Actinomyces*. Accurate diagnosis is made by direct microscopic observation, culture techniques, and serological tests.

Fungi can be observed directly in clinical specimens such as tissues, sputum, bronchial washings, hair, or skin scrapings. The specimens are usually treated with 10% potassium hydroxide and heated to destroy host cellular elements, leaving the KOH-resistant cell wall of the fungal agent unaltered. The structures (conidia, conidiophores) of many fungi are distinctive enough in the vegetative phase to permit presumptive diagnosis. Dried and fixed films may be stained with Gram's stain, periodic acid–Schiff stain, or Wright stain. The microscopic examination of filamentous molds can be performed by teasing apart a portion of the colony on a microscopic slide and adding a drop of lactophenol aniline blue stain.

Specimens are also cultured on laboratory media, even when direct examination provides almost positive results. Sabouraud agar and richer media are used with and without antibiotics to inhibit contaminating bacterial species. Growth at room temperature and at 37°C can be used to distinguish the yeast and mycelial phases of dimorphic fungi. Slide cultures can be made to observe fungal structures in their natural state, but this is not recommended for those agents of systemic mycoses that can cause fatal disease.

Serological techniques have value in the diagnosis and prognosis of some fungal diseases; however, most diagnoses rely on macroscopical or microscopical examination.

IMMUNITY

Antibody is regarded as being less important than cell-mediated immunity as a protective device in response to fungal infections. This is best illustrated by the variety of fungal infections found in patients with defective T lymphocytes. In contrast, defects in B cells do not usually predispose the patient to fungal infections. Studies to date indicate that antibodies are protective only within a very narrow range of conditions in experimental animals and with only a few pathogens such as *Candida* and *Cryptococcus*. Antibodies act primarily to enhance the efficiency of the cell-mediated response. Those fungi that are allergenic when inhaled, such as *Alternaria* and *Cladosporium*, elicit an IgE-mediated hypersensitivity. The immune response triggered by these allergenic fungi is evidenced by enhanced activities by macrophages and lymphocytes. Again the morphology of the fungal agent in tissue influences the host response. Conidia of *Aspergillus fumigatus*, for example, are eliminated by macrophages, but neutrophils are more destructive to hyphae.

The majority of fungal pathogens induce a delayed type of hypersensitivity, and skin tests are available for determining one's exposure to the fungal agent.

Table 35-1. Industrial Products Obtained Through the Activities of Fungi

Organism	Product
VITAMINS	
Eremothecium ashbyi	Riboflavin
FOOD AND BEVERAGES	
Saccharomyces cerevisiae	Wine, ale, sake, baker's yeast
Penicillium species	Varieties of cheese
Saccharomyces rouxii	Soy sauce
Saccharomyces carlsbergensis	Lager beer
ORGANIC ACIDS AND SOLVENTS	
Aspergillus niger	Citric acid and gluconic acid
Saccharomyces cerevisiae	Ethanol from glucose
SINGLE-CELL PROTEINS	
Saccharomyces lipolytica	Microbial protein from petroleum alkanes
Candida utilis	Microbial protein from paper pulp waste
PHARMACEUTICALS	
Penicillium chrysogenum	Penicillins
Cephalosporium acremonium	Cephalosporins
Rhizopus nigricans	Steroid transformation

TREATMENT AND PREVENTION

The fungi are eukaryotic cells with cell walls that do not contain peptidoglycan. The antimicrobial agents that affect bacterial cells, for example, cell wall inhibitors such as penicillin, have no effect on fungal cells. Systemic fungal diseases are the most life threatening, but very few antimicrobials have been developed that can affect their outcome. Most antifungal agents are toxic, have insufficient solubility in the host, and are poorly adsorbed. The antifungal agents are discussed in Chapter 11.

Fungal surface components for the most part are poorly antigenic. Vaccine development to prevent fungal infections has not been actively pursued. In addition vaccination would be impractical because of the rarity of mycoses in the general public.

IMPORTANCE TO HUMANS

The fungi play a role in disease but, even more important, they are also of value in nature and industry. Fungi, because of highly developed enzymatic systems, can degrade organic material, a property that may be of immeasurable value or detriment to humans. Fungi are important in the recycling of carbon and other elements. In addition they can degrade recalcitrant molecules and have an important role in the disposal of chemical wastes. Their metabolic activities are also responsible for the destruction of many grains, fabrics, and foods. Fungal contamination of grains, peanut crops, and fruits (apples) can lead to the production of *mycotoxins* that cause serious illness in humans and various domestic animals (mycotoxins are discussed later in the chapter). Fungi play an important role in industry because they are used in the preparation of a variety of products beneficial to humans (Table 35-1), such as antibiotics, the most famous of which is penicillin produced by the green mold *Penicillium*. Fungi such as mushrooms, truffles, and morels are also prized food delicacies.

CLASSIFICATION

Modern taxonomists place the fungi in the kingdom Fungi, making it distinct from the animal and plant kingdoms. The kingdom Fungi is separated into two divisions, Myxomycota and Eumycota. The Myxomycota, or slime molds, are nonpathogenic, while the Eumycota are the principal pathogens for humans. The Eumycota can be separated into five subdivisions, four of which are pathogenic to humans:

1. *Zygomycotina*. The Zygomycotina are the most primitive members of the fungi. They are filamentous and lack septa, a characteristic that separates them from other subdivisions. They reproduce sexually and asexually. The asexual spores or sporangiospores are produced in a sac called a *sporangium*. The members of this group are not primary pathogens but are opportunistic

in certain predisposed patients.

2. *Ascomycotina*. The Ascomycotina produce asexual and sexual spores. The asexual spores are called *conidia*, and the sexual spores produced by those species of medical interest are called *ascospores*. Some members of this group (dermatophytes) cause skin infections, while others are pathogenic yeasts.
3. *Basidiomycotina*. The Basidiomycotina produce septate hyphae and sexual spores called *basidiospores*. Mushrooms and puffballs belong to this subdivision.
4. *Deuteromycotina* (Fungi Imperfecti). The Deuteromycotina reproduce asexually. Sexual reproductive structures are unknown. Many species pathogenic to humans belong to this group.

FUNGAL DISEASES

SYSTEMIC MYCOSES

The agents of systemic mycoses are dimorphic fungi found in the soil and are acquired by humans by inhalation of spores. Initially there is a pulmonary infection, which may or may not be symptomatic, but eventually the organism is disseminated to other organs. The systemic mycoses to be discussed are histoplasmosis, coccidioidomycosis, blastomycosis, paracoccidioidomycosis, and cryptococcosis.

Histoplasmosis

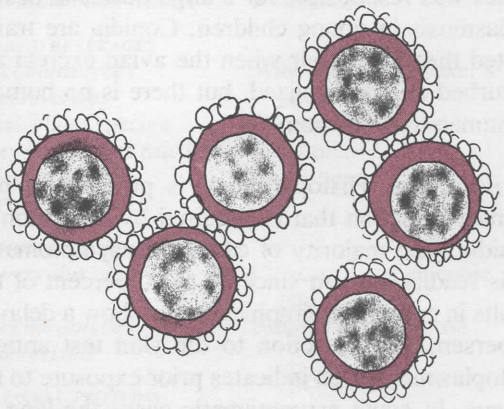
Histoplasma capsulatum is the causal agent of histoplasmosis. Since the discovery of a sexual stage (asci and ascospores when properly noted) the perfect stage is classified as *Emmonsiella capsulatum*, but we will retain its original name. Histoplasmosis occurs worldwide. In the United States the incidence of infection is highest in the Mississippi Valley. Several million people in the United States are infected by *H. capsulatum*, but fewer than 5 percent are symptomatic.

EPIDEMIOLOGY. *H. capsulatum* is found in soil that is nitrogen-enriched, especially soils containing high levels of avian fecal material. The organism can easily be isolated from excrement in chicken houses and starling roosts and from guano in bat caves. The roosting of several hundred thousand starlings in a small midwestern town in the United States was responsible for a large outbreak of histoplasmosis in young children. Conidia are transmitted through the air when the avian excreta are disturbed and aerosolized, but there is no human-to-human transmission.

PATHOGENESIS. Histoplasmosis is primarily a pulmonary infection that is acquired by inhalation of conidia. The majority of cases are asymptomatic, as is readily evident since 60 to 90 percent of the adults in certain geographical areas show a delayed hypersensitivity reaction to the skin test antigen histoplasmin, which indicates prior exposure to the fungus. In acute asymptomatic cases the lung lesion may heal with calcification, a condition that resembles tuberculosis lesions. Pulmonary histoplasmosis sometimes becomes chronic and is almost indistinguishable from tuberculosis. Reactivation of the lesions is more frequent where the disease is endemic. Untreated chronic disease may lead to dissemination of the organism. Disseminated histoplasmosis is characterized by an invasion of the entire reticuloendothelial system. In areas where histoplasmosis is endemic, disseminated histoplasmosis occurs 400 times more often among leukemic patients than the rest of the population. The symptoms include fever plus liver, spleen, and lymph node enlargement. The phagocytic cells found in these tissues are gorged with small oval yeast cells, and the organs display small granulomas similar to those produced in miliary tuberculosis. In approximately 50 percent of individuals infected, mucocutaneous lesions of the oral cavity appear. The ulcerative oral lesions are often painful and are accompanied by regional lymph node enlargement.

LABORATORY DIAGNOSIS. Sputum and gastric washings can be stained and examined for *H. capsulatum* in the benign stage of the disease. Direct fluorescent antibody technique can also be applied to

Fig. 35-12. *Histoplasma capsulatum*. A. Diagram of tuberculate macroconidia, which appear as round, thick-walled spores. Each spore has finger-like projections (tubercles) attached to it. B. Photomicrograph of cultured *H. capsulatum* (Courtesy G. Roberts.)



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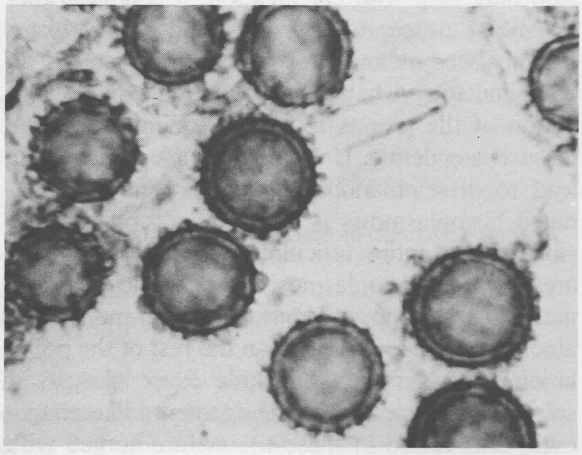


B

clinical specimens for rapid identification. Because of the relatively few fungal cells in host specimens, however, microscopical identification of *H. capsulatum* in the benign stage is difficult if not impossible. During the disseminated stage of the mycosis, sputum, urine, bone marrow, and blood can be examined for the presence of the fungus. Staining these specimens with Giemsa's or Wright's stain reveals the fungus as small, oval, yeast-like cells crowded within the macrophages. Clinical specimens should also be inoculated onto Sabouraud agar with and without antibiotics at room temperature and observed for 1–2 weeks for the appearance of mycelial growth and the characteristic tuberculate conidia that develop from the hyphae (Fig. 35-12). Since the organism is dimorphic the yeast phase can be demonstrated by cultivation on enriched media such as brain-heart infusion or yeast extract containing antibiotics (penicillin and streptomycin) at 37°C.

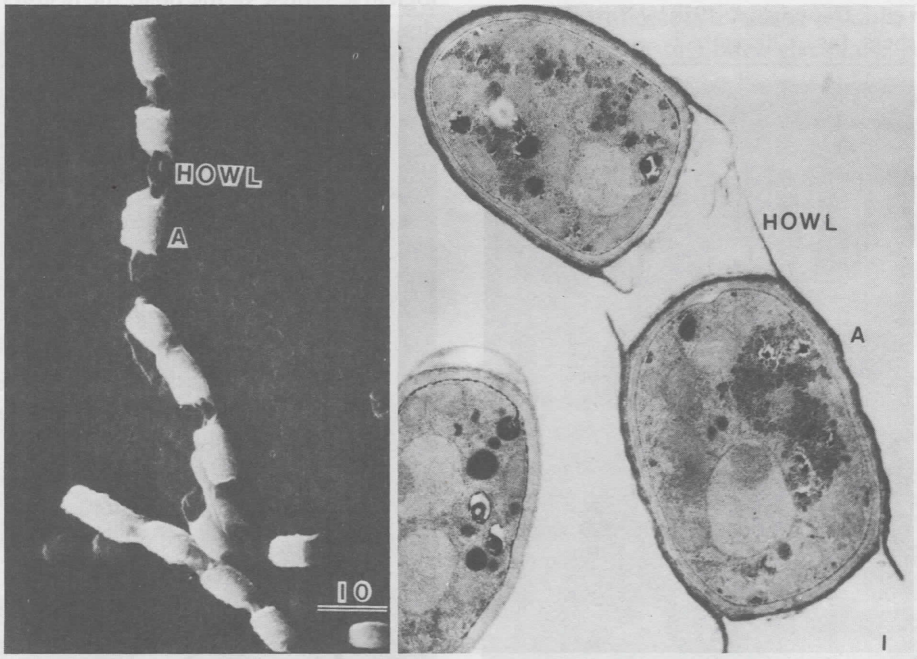
Definitive diagnosis of histoplasmosis is established by serological methods. Complement fixation tests have been used but are difficult to evaluate and are expensive. High antibody titers to histoplasmin, the skin test antigen, may also be present in cases of cryptococcosis and blastomycosis. Moreover, histoplasmin, which is injected intradermally to test for sensitivity to the fungus before serodiagnosis, increases the titer of complement fixation antibodies and makes the test unreliable. In disseminated histoplasmosis the immunological tests may be negative. The immunodiffusion test can be used on sera of patients who have or have not been skin-tested. In the test the patient's serum interacts with concentrated histoplasmin antigen, and a number of precipitin bands are formed, one of which is not influenced by previous skin testing.

TREATMENT. Histoplasmosis is now considered a common benign infection rather than a rare fatal disease. Successful treatment of disseminated histoplasmosis is still dependent on early diagnosis. Amphotericin B, administered intravenously (IV) slowly over a period of 6–8 hours, has been the best means of treatment. Such treatment must be repeated for several weeks. Nephrotoxicity is a problem with amphotericin B; therefore blood urea nitrogen or blood creatinine tests and urea clearance tests are essential.

Coccidioidomycosis

Coccidioidomycosis is caused by the agent *Coccidioides immitis*. Disease is initiated as an upper respiratory infection that usually resolves on its own,

Fig. 35-13. *Coccidioides immitis*. A. Scanning electron micrograph of arthroconidia alternating with lysed vegetative component of the hypha. (HOWL = hyphal outer wall; A = arthroconidium; bar = 10 μm). B. Thin-section electron micrograph of a portion of A. (From M. Hupert et al. in D. Schlesinger [Ed.], *Microbiology—1983*. Washington, DC: American Society for Microbiology, 1983.)



A B

provided a cell-mediated immune response to the fungus is mounted by the patient. Dissemination of the agent, heralded by high antibody titers and absence of a cell-mediated immune response, often leads to a fatal outcome.

EPIDEMIOLOGY. Coccidioidomycosis is a highly endemic disease associated with hot, semiarid areas. In the United States the disease is endemic in areas of southern California, Arizona, New Mexico, and Texas. The fungus lives in the soil in a mycelium-arthroconidia (arthrospores) cycle. Autolysis of mycelia releases arthroconidia (Fig. 35-13), which are dispersed in the air as dust aerosols and inhaled by humans frequently during dust storms or during building construction or excavation. Disseminated disease occurs more frequently among persons with pigmented skin: blacks, Filipinos, and American Indians. The fatality rate among American Indians is three to five times that of Caucasians, for reasons that are not immediately apparent.

PATHOGENESIS. After their deposition on pulmonary surfaces, the arthroconidia are first attacked by neutrophils and later by macrophages. Most infections are asymptomatic or produce mild pulmonary symptoms that resolve within a few weeks. These infected individuals are sensitive to coccidioidin, the fungal antigen, when the latter is injected intradermally as evidenced by a delayed hypersensitivity reaction, heralding, as in primary histoplasmosis, the emergence of a cell-mediated immune response and a good prognosis. The principal symptoms of infection are fever, chest

Fig. 35-14. Coccidioidomycosis. Disseminated disease with granulomatous crusted lesions of the face and nose. (Photograph by A. Gregerson, F.B.P.A. Reproduced with permission from G. M. Lewis, M. E. Hopper, J. W. Wilson, and O. A. Plunkett, *An Introduction to Medical Mycology* [4th ed.]. Copyright © 1958 by Year Book Medical Publishers, Inc., Chicago.)

pain, cough, and other flu-like symptoms. Circumscribed nodules are observed by x-ray in symptomatic disease. Allergic responses are also observed in symptomatic disease. The pulmonary lesion may be a source of dissemination of the fungus with serious consequences. Disseminated disease is usually associated with an overwhelming exposure to fungal spores. The lungs, meninges, liver, kidneys, spleen, and deep lymph nodes are the major organs affected. Meninges involvement usually results in a rapidly fatal course, particularly for dark-skinned individuals. Disseminated lesions may also appear on the skin, subcutaneous tissue, and bones (Fig. 35-14).

LABORATORY DIAGNOSIS. Depending on the immunological response of the host, the lesions found in the disseminated disease may be suppurative or granulomatous. Coccidioidomycosis resembles many other granulomatous diseases, and accurate diagnosis is based on laboratory identification of the fungus in pathological tissue in culture or by serological tests. *C. immitis* is a dimorphic fungus. The inhaled arthrospore is transformed into a large spherule in its tissue phase of development. Invagination of the inner layers of the spherule segments the protoplasm into smaller units or *endospores* (Fig. 33-15). Clinical specimens of sputum, pus, or tissue can be examined for the presence of spherules. Clinical specimens should also be cultivated on Sabouraud and blood agar to demonstrate the mycelial phase of the organism. Arthrospores are easily disseminated; therefore laboratory techniques should be performed under a safety hood.

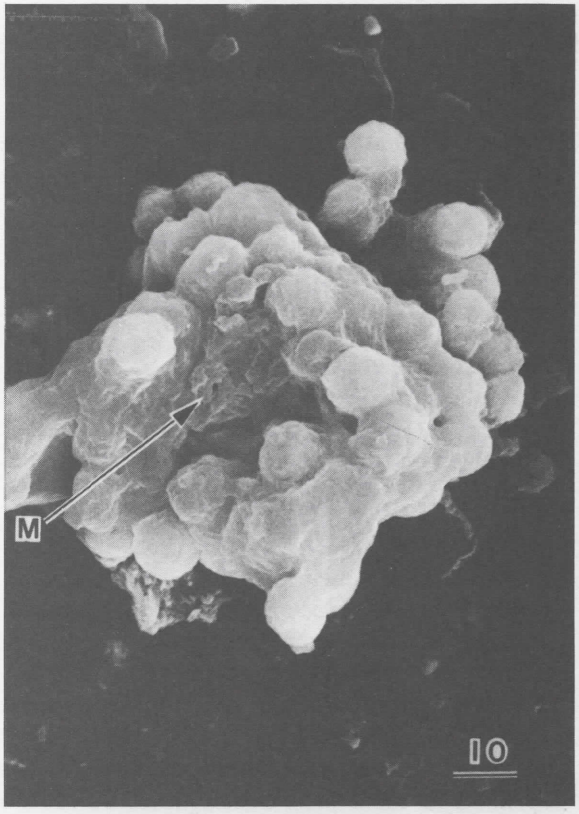
Skin test data using coccidioidin is not considered diagnostic, but if the test is negative it will help to exclude coccidioidomycosis.

Serological testing is an invaluable tool is diagnosis and prognosis. IgM antibodies are produced early in disease and can be detected by tube precipitin tests, latex agglutination, and immunodiffusion. IgG antibodies persist longer than do IgM antibodies and can be detected by complement fixation, counterimmunoelectrophoresis, and immunodiffusion. The titers detected in these tests reflect whether there is active infection or the disease is in the convalescent stage.

Recent studies indicate a relationship between tissue eosinophils and the severity of disease. The greatest degree of peripheral and tissue eosinophilia is observed in patients with disseminated disease, but there is an apparent absence of eosinophils in those with pulmonary nodules.

TREATMENT. To date, amphotericin B administered IV is the most effective antifungal agent in the treatment of coccidioidomycosis. A formalin-

Fig. 35-15. Scanning electron micrograph of a cluster of endospores of *Coccidioides immitis* held within a spherule membrane (*M*) (bar = 10 μm). From M. Hupert et al. In D. Schlesinger, [Ed.], *Microbiology—1983*. Washington, D.C.: American Society for Microbiology, 1983.)



killed vaccine derived from the spherule-endospore phase is currently being tested in human subjects.

Blastomycosis

Blastomycosis is caused by *Blastomyces dermatitidis*, a thermally dimorphic fungus that initiates disease by infecting the lung. The disease is chronic and is characterized by granulomatous lesions that may give rise to a disseminated form.

EPIDEMIOLOGY. Blastomycosis appears to be endemic in southern Canada and in central midwest, southeast, and Atlantic coastal states of the United States. The organism has not, except for two or three instances, been isolated from the soil. The fungus is believed to remain dormant in the soil for most of the year and is then stimulated by climatic conditions to reproduce. The incidence of infection has been difficult to evaluate because antigen available for skin testing often gives false-positives. The incidence of disease is highest in blacks and in men, particularly those over 50 years of age, again for reasons unknown.

Most infections are believed to occur by inhalation of disseminated spores, but there are a few recorded cases of blastomycosis having been acquired by contact with fomites.

PATHOGENESIS. Blastomycosis generally takes two forms: pulmonary with dissemination, and chronic cutaneous disease. Pulmonary disease in many patients causes mild respiratory disease but is seldom acute. Pulmonary lesions may heal, but the organisms are usually disseminated and involve the skin. Chronic cutaneous disease is characterized by subcutaneous lesions that ulcerate. The lesions are found predominantly on the face, hands, and lower legs, or mucocutaneous areas such as the larynx (Fig. 35-16). Untreated, the disease progresses and involves many unexposed areas of the body. The lesions are raised, crusty, and discolored. When the lesions heal they leave deforming scars that cover the face, neck, or other areas. Systemic blastomycosis occurs from unresolved infection but is rare. Disseminated disease involves most organs and may also be manifested as cutaneous or osseous (bone) lesions.

LABORATORY DIAGNOSIS. *B. dermatitidis* in the yeast phase exists as a spherical cell 8 to 15 μm in diameter. When observed in stained sections or preparations the cytoplasmic membrane of the fungus is seen to shrink and the cell appears double walled. In addition the buds of the dividing yeast remain attached to the parent cell, giving the appearance of a figure eight (Fig. 35-17).

Skin scrapings, sputum, and pus should be examined for typical thick-walled yeast forms. The features of the yeast are pathognomonic for blasto-

Fig. 35-16. Blastomycosis. Advanced systemic and cutaneous disease. (From C. W. Emmons et al., *Medical Mycology* [3rd ed.]. Philadelphia: Lea & Febiger, 1977.)

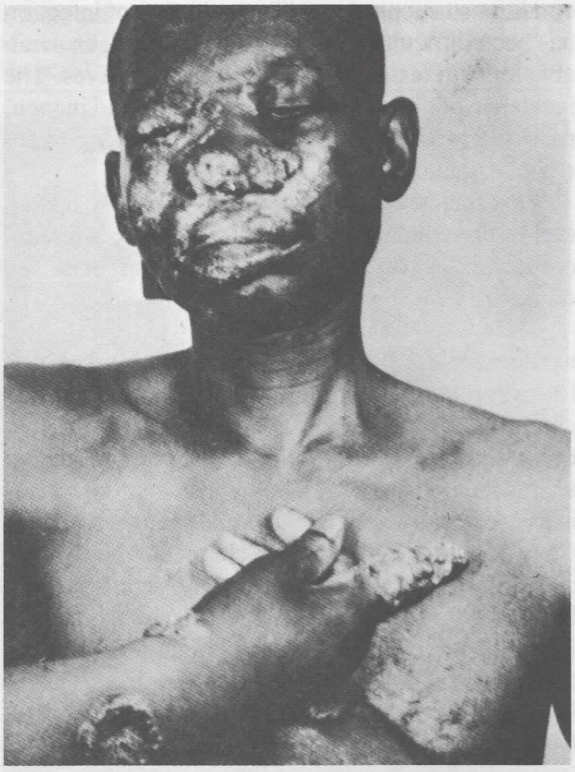
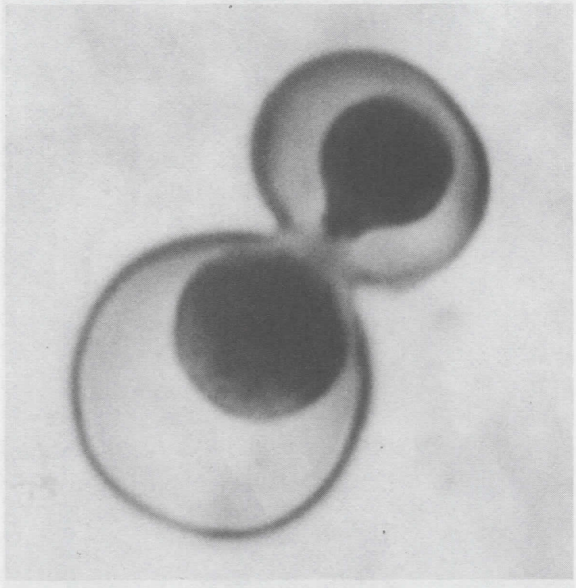


Fig. 35-17. Stained wet preparation of *Blastomyces dermatitidis*. The membrane shrinks during the staining, giving the appearance of a double wall. The smaller bud remains attached to the mother cell, which gives the appearance of a figure eight. (From H. W. Larsh and N. L. Goodman. Fungi of Systemic Mycoses. In E. H. Lennette, A. Balows, W. J. Hausler, Jr., and J. P. Truant [Eds.], *Manual of Clinical Microbiology* [3rd ed.]. Washington, DC: American Society for Microbiology, 1980.)



mycosis. Specimens should also be cultured on Sabouraud and enriched media such as brain-heart infusion to detect the mycelial as well as the yeast phase of the organism. The most useful serological test is an immunodiffusion test. The development of specific precipitin lines is indicative of disease. A decline or disappearance of these precipitin lines is evidence of a favorable prognosis.

TREATMENT. Cutaneous blastomycosis is never fatal, but untreated lesions may last for years. Systemic blastomycosis, if left untreated, is almost invariably fatal. Administration of hydroxystilbamidine isethionate over a 30-day period has proved successful. Although amphotericin B is apparently more effective, the nephrotoxic effect of the drug must be considered if it is used for prolonged periods.

Paracoccidioidomycosis

Paracoccidioidomycosis, also called *South American blastomycosis*, is a chronic, sometimes fatal disease characterized by lesions of the mucous membranes of the mouth. It is seldom seen in the United States. The causal agent of the disease is *Paracoccidioides brasiliensis*.

EPIDEMIOLOGY. *P. brasiliensis* is believed to be an inhabitant of the soil but has been only occasionally isolated from the soil. Most cases of disease have been reported from Brazil, Venezuela, Argentina, and other South American countries. The highest

incidence of disease occurs among agricultural workers.

PATHOGENESIS. Paracoccidioidomycosis is an endemic disease whose mode of infection is not known. Many investigators believe that the respiratory tract and lungs are the first sites of infection; however, oral lesions and enlargement of regional lymph nodes are the first symptoms to be recognized. The oral lesions are granular but seldom ulcerative. The infection may become progressive, with development of lesions elsewhere on the body, including the skin, lymph nodes, and lungs. In the infected tissue a granuloma, similar to the granulomas encountered in tuberculosis is the predominant pathological characteristic. In clinical specimens fungi may be found outside or inside phagocytic cells as oval, multiple budding cells averaging between 25 and 30 μm in diameter. Prognosis for the disseminated form of the disease is very poor, the fatality rate reaching 50 percent.

LABORATORY DIAGNOSIS. Clinical diagnosis can be made from the appearance of oral lesions, but confirmation depends on the finding of fungal parasites in clinical specimens. Procedures for the laboratory diagnosis are the same as those employed in North American blastomycosis. The yeast phase cells show multiple buds much resembling a ship's wheel, which distinguishes them from *B. dermatitidis*. The mycelial phase and characteristic conidia cannot be distinguished from those of *B. dermatitidis*. Serological tests are not totally reliable; however, the immunodiffusion, counterelectrophoresis, and complement fixation tests are reasonably specific.

TREATMENT. The sulfonamides, such as sulfadiazine and sulfisoxazole, have proved effective in treatment. Treatment must be continued for approximately 2 years to prevent relapses. If the fungal species encountered in the infection is resistant to sulfonamides, amphotericin B may be substituted.

Cryptococcosis

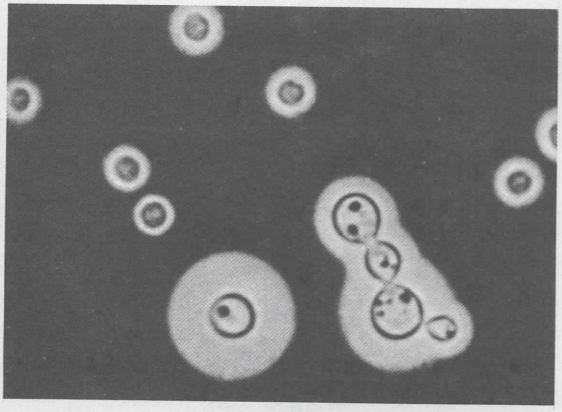
Cryptococcosis is a subacute chronic infection that involves mainly the lungs, brain, and meninges.

Fig. 35-18. Cryptococcosis. Extensive cutaneous involvement. The gelatinous material represents fungus in nearly pure culture. (Courtesy A. C. Curtis, Ann Arbor, MI. Reproduced with permission from G. M. Lewis, M. E. Hopper, J. W. Wilson, and O. A. Plunkett, *An Introduction to Medical Mycology* [4th ed.]. Copyright © 1958 by Year Book Medical Publishers, Inc., Chicago.)

The causative agent, *Cryptococcus neoformans*, has as its primary reservoir the soil where pigeon droppings containing organic nitrogen (urea) favor the growth of yeasts. It is capable of rapid growth and development in the feces. The fungus is not dimorphic, and only the yeast phase exists in the saprophytic and parasitic states. Cryptococcosis has no geographical limitations.

PATHOGENESIS. *Cryptococcus* infection occurs more frequently in patients seriously debilitated by such conditions as Hodgkin's disease, tuberculosis, and various lymphomas than in healthy persons. The disease is believed to begin as a pulmonary infec-

Fig. 35-19. *Cryptococcus neoformans*. A nigrosin-stained wet preparation of spinal fluid showing oval fungal cells surrounded by a clear capsule. (From M. Silva-Hunter and B. H. Cooper. Medically Important Yeasts. In E. Lennette, A. Balows, W. J. Hausler, Jr., and J. P. Truant [Eds.], *Manual of Clinical Microbiology* [3rd ed.]. Washington, DC: American Society for Microbiology, 1980.)



tion that seldom goes beyond the subclinical stage. In many patients the pulmonary lesion heals without calcification and goes unrecognized. Systemic manifestations in which the central nervous system or any organ of the body may become involved occasionally follow pulmonary infection. Central nervous system involvement is characterized by the development of granulomatous lesions of the meninges; left untreated, they are usually fatal. Cutaneous lesions manifested as papules or abscesses may also be the result of systemic infection and can appear anywhere on the body (Fig. 35-18).

LABORATORY DIAGNOSIS. India ink or nigrosin preparations of sputum, pus, urine, or infected tissue reveal *C. neoformans* as an oval, thick-walled yeast cell surrounded by a wide clear capsule (Fig. 35-19). After several days of incubation on Sabouraud agar, a slimy, mucoid, creamy colony containing budding cells with large capsules but no hyphae develops. *C. neoformans* can be differentiated from other *Cryptococcus* species by the former's (1) ability to grow at 37°C, (2) production of brown colonies on birdseed agar, (3) ability to assimilate specific sugars, (4) pathogenicity for animals, and (5) urease activity.

Fig. 35-20. Chromomycosis. (Courtesy G. Roberts.)

TREATMENT. Cryptococcal meningitis is treated with amphotericin B plus flucytosine, but there is a failure rate of over 40 percent if one employs only flucytosine. Both drugs are toxic, especially flucytosine, in patients with renal disease. Ketoconazole administered with subtherapeutic doses of amphotericin B has been demonstrated to increase the killing of *Cryptococcus*.

SUBCUTANEOUS MYCOSES

Subcutaneous mycotic infections are those that remain localized in subcutaneous tissue. Most infections are the result of puncture wounds by objects contaminated by fungal species found in the soil. Several agents are associated with subcutaneous mycoses. We will elaborate on only one, *Sporothrix schenckii*, the most frequently associated with infection. Subcutaneous diseases caused by other fungal agents are outlined in Table 35-2.

Sporotrichosis and Other Subcutaneous Mycoses

Sporotrichosis is a subcutaneous infection that is seen most often in greenhouse workers, farmers, and others who are constantly exposed to the soil

Fig. 35-21. Lobomycosis. Keloidal lesions of 30 years' duration. (From C. W. Emmons, et al., *Medical Mycology* [3rd ed.]. Philadelphia: Lea & Febiger, 1977.)

(see boxed essay, p. 799). The causative agent, *Sporothrix schenckii*, is found in the soil and vegetation and has a worldwide distribution. Sporotrichosis is endemic in parts of Brazil, South Africa, and Zimbabwe.

PATHOGENESIS. Cutaneous infection resulting from traumatization of tissue and contact with spores begins as a small, purplish, ulcerated lesion. The lesion becomes necrotic and soon spreads subcutaneously to local lymph nodes, causing them to become swollen and sometimes necrotic (Fig. 35-22). The subcutaneous infection remains localized and seldom becomes disseminated. Ulcerated lesions may heal, but new ones appear nearby. Infection in areas where sporotrichosis is endemic often results in a single lesion at the original site of inoculation (fixed sporotrichosis). These lesions may appear to heal spontaneously but usually reappear at the same location. Pulmonary disease is being increasingly recognized in hospitalized patients who inhale these spores. Like the systemic mycoses, pulmonary infections by *S. schenckii* mimic tuberculosis. Disseminated sporotrichosis is seldom observed but when it occurs usually involves the skeletal system and seldom organ systems.

LABORATORY DIAGNOSIS. Aspirated pus from lesions should be cultured on Sabouraud agar or brain-heart infusion agar at 37°C to demonstrate the yeast phase and on Sabouraud agar at room temperature to demonstrate the mycelial phase. The yeast phase cells are oval and sometimes cigar shaped. The mycelial phase is characterized by brown to black colonies in which conidia appear as flower-like clusters on conidiophores (Fig. 35-23).

The slide latex agglutination test is the preferred technique for serological diagnosis. An increasing or sustained titer is helpful in diagnosing pulmonary disease. The test is useful for monitoring disease but is of little prognostic value.

TREATMENT. Saturated solutions of potassium iodide in milk administered orally are the best treatment of the ulceroglandular type of sporotrichosis. Intravenous amphotericin B therapy is used in the treatment of localized pulmonary and disseminated sporotrichosis.

DERMATOPHYTOSES

General Characteristics

The fungi that invade the keratinized and cutaneous areas of the body (nails, hair, and skin) are called the *dermatophytes*. The dermatophytes are represented by three major genera of pathogenic molds: *Microsporum, Trichophyton*, and *Epidermophyton*. In the United States, *Trichophyton rubrum* is the most frequently encountered causal agent of cutaneous mycoses. The diseases pro-

Table 35-2. Characteristics of the Subcutaneous Mycoses and Their Causal Agents

Disease	Agent	Clinical manifestations	Epidemiology	Treatment
Sporotrichosis	*Sporothrix schenckii*	Nodules along lymphatic channels that may ulcerate or superficial lesions not involving lymphatics; extracutaneous infections rare	Infection highest among agricultural workers	Potassium iodide
Chromomycosis (Fig. 35-20)	*Phialophora verrucosa, Cladosporium carrioni, Fonsecaea* species, *Wangiella dermatitidis*	Infections usually on lower extremities with discolored lesions that become raised and cauliflower-like if untreated; systemic invasion rare	Predominant in tropical and subtropical regions among barefoot agriculturalists	Surgery in early disease, flucytosine for advanced cases
Mycetoma	*Petriellidium boydii, Madurella mycetomatis, Madurella grisea, Acremonium kiliense*, and others	Granulomatous lesions with multiple sinus tracts; feet, hands, and other exposed areas usually infected; scar tissue leads to disfigurement of affected area	Found worldwide but most frequently in Africa, Asia, and tropical and subtropical areas	Surgery to remove tissue that prevents drug penetration; nystatin, amphotericin B, and flucytosine
Rhinosporidiosis	*Rhinosporidium seeberi*	Lesions of mucous membranes of nose or soft palate; lesions become enlarged masses that can interfere with breathing	Found primarily in India and Sri Lanka and mostly in males	Surgery
Lobomycosis (Fig. 35-21)	*Loboa loboi*	Lesions localized primarily on feet, legs, or face; lesions spread as enlarged masses	Found primarily in Amazon basin	Surgery and sulfa drugs
Subcutaneous phycomycosis	*Basidiobolus haptosporus*	Nodule formation in subcutaneous tissue; infection usually in abdomen or a limb	Found primarily in Africa among children 5–9 years of age	Potassium iodide

Fig. 35-22. Necrotic cutaneous lesions caused by *Sporothrix schenckii*. (Courtesy G. Roberts.)

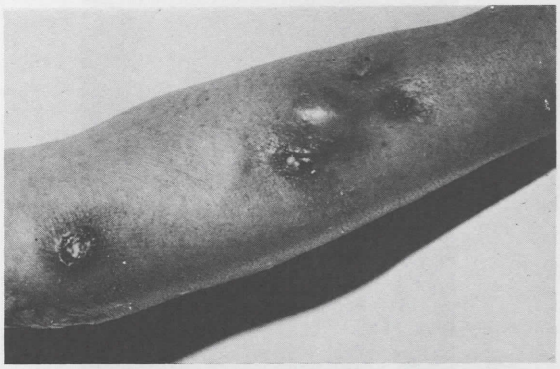
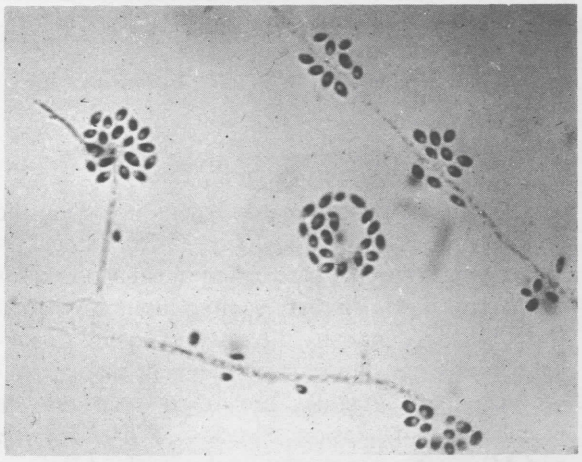


Fig. 35-23. Floweret arrangement of conidia on conidiophores of *Sporothrix schenckii*. (Courtesy G. Roberts.)



duced by these three genera are referred to as *tineas* (*ringworm*) of various parts of the body. Tinea capitis is ringworm of the scalp; tinea barbae, ringworm of the beard area; tinea pedis, ringworm of the feet; tinea cruris, ringworm of the groin; tinea corporis, ringworm of the body; tinea favosa, ringworm of the scalp; tinea unguium, ringworm of the nails; and tinea imbricata, ringworm of the torso.

Dermatophytes produce enzymes that enable them to degrade keratinized tissue. These enzymes include keratinase, elastase, and collagenase, which break down keratin, elastin, and collagen, respectively, all of which are components of epithelial and connective tissue. The reason for the inability of dermatophytes to invade deep tissue is not known but may be related to temperature, tissue conditions, oxidation-reduction potential, and other factors.

Dermatophytes abound worldwide as saprophytes in the soil, but many of them have evolved to a parasitic existence. Some dermatophytes are strict parasites of humans and are no longer found in the soil. Others no longer found in the soil are parasites of lower animals but can infect humans. Finally some are found free in nature and accidentally cause infections in humans. The cutaneous mycoses are the only contagious fungous diseases in humans.

Laboratory Diagnosis

Dermatophytic infections of the hair, nails, and skin are sometimes diagnosed from the clinical symptoms—loss of hair, thickened and discolored nails, or generalized scaling. In ringworm of the scalp the patient can be examined directly for skin lesions or areas where there is loss of hair. In some scalp infections, particularly those caused by *Microsporum*, the infected hair fluoresces a bright yellow-green when exposed to Wood's lamp (ultraviolet). Hair, skin scrapings, or nail clippings can be examined directly if the infected material is placed on slides and 10% potassium hydroxide (KOH) is added. The preparation is gently heated to free the hyphae from keratinous material. The infected material is examined for the presence of septate hyphae or arthrospores. Identification of the specific dermatophyte can be accomplished by culturing the infected material on Sabouraud agar and examining the macroscopical colonial characteristics as well as the microscopical morphology of individual species. The Sabouraud agar should contain cycloheximide (Actidione), which suppresses growth of most saprophytic fungi but not dermatophytes, and an antibacterial agent such as chlortetracycline hydrochloride (Aureomycin) or chloramphenicol to

Fig. 35-24. Tinea capitis. (Courtesy G. Roberts.)

give the slower-growing dermatophytes a chance to outgrow bacteria.

Treatment

Griseofulvin given orally has proved useful for eliminating scalp infections. For body infections, several antifungal ointments containing the following agents can be applied: 3% salicylic acid, 5% undecylenic acid, 5% benzoic acid, or 5% sodium thiosulfate. For foot infections, potassium permanganate (1:4000), 20% zinc undecylenate, 3% salicylic acid, or 5% benzoic acid are helpful in treatment. Topical antibiotics such as tolnaftate, miconazole nitrate, and clotrimazole are also available.

Tinea Capitis (Ringworm of the Scalp)

Most species of *Microsporum* and *Trichophyton* may be involved in ringworm of the scalp (Fig. 35-24). *M. canis*, transmitted by infected dogs and cats, is a major causal agent of this disease. The infection is usually acquired during childhood and begins with inflammation and itching of the scalp. The fungi spread on the keratinized areas of the scalp and may even involve the hair shaft, so that hair is broken off at the scalp level. If the hyphae are found growing only within the hair shaft, the infection is called *endothrix* infection. If the hyphae are found both within and on the external surface of the hair, the infection is called *ectothrix* infection.

Fig. 35-25. Tinea corporis. (Courtesy G. Roberts.)

Tinea Corporis (Ringworm of the Body)

Tinea corporis is a dermatophytosis involving the nonhairy areas of the body (Fig. 35-25). Fungal infections of the face are often misdiagnosed as connective tissue diseases such as lupus erythematosus or disorders caused by overexposure to sunlight. The lesions appear as reddened, scaly, papular eruptions. Pustules are often seen on the periphery of the lesion. Species of *Trichophyton* and *Microsporum* are commonly involved in tinea corporis infections.

Tinea Pedis (Ringworm of the Feet)

Tinea pedis, usually called *athlete's foot*, is believed by many investigators to be spread primarily through the use of public showers, swimming pools, or other such facilities and is favored by failure to dry one's feet between the toes after bathing. The disease is characterized by itching and burning between the toes. Lesions releasing a watery fluid

SPOROTRICHOSIS ASSOCIATED WITH WISCONSIN SPHAGNUM MOSS

On February 2, 1981, the Wisconsin Division of Health, Bureau of Community Health and Prevention (BCHP), was notified of a diagnosis of sporotrichosis in two brothers employed at a garden center in southeastern Wisconsin. An investigation of the garden center by the BCHP revealed two additional cases of sporotrichosis among the employees. All four cases involved male high school students, aged 16 and 17, who worked part-time at the garden center. Each had developed an ulcerating lesion on his hand or wrist during December 1980 and was initially treated with antibiotics without resolution. In three of the cases, nontender ascending lymphangitis subsequently developed. Swabs of the lesions were sent to local hospital laboratories for fungus culture; two were positive for *Sporothrix schenckii*. All four individuals were successfully treated with potassium iodide.

The garden center consists of a retail store and wholesale greenhouse and employs approximately 80 people. Sixteen workers made approximately 1000 gravesprays (a decorative winter covering for grave sites, constructed by weaving balsam boughs through a matting of water-soaked sphagnum moss) during the months of November and December 1980. The sphagnum moss used was harvested from bogs located in central Wisconsin and was purchased from one wholesale dealer.

Of seven male workers regularly involved in making gravesprays, four developed sporotrichosis. Of three additional male employees who occasionally helped with the production of gravesprays and six female employees who were involved in final decorative preparations, none developed sporotrichosis. In an investigation of a local cemetery where 12 employees produce 2000 to 3000 gravesprays per autumn using sphagnum moss purchased from the same Wisconsin supplier, no additional cases were noted. Samples of the sphagnum moss used in December during the gravespray production at the garden center and tested at the mycology laboratory, Wisconsin State Laboratory of Hygiene, were culture-positive for *S. schenckii*.

Sporotrichosis is a well-described, yet uncommon, chronic infection caused by *S. schenckii*, a dimorphic fungus present worldwide in soil, plants, and decaying vegetation. Some of the outbreaks reported among florists and nursery and forestry workers have been associated with sphagnum moss grown in Wisconsin. Central Wisconsin, one of the largest areas of sphagnum moss production in the nation, supplies moss for horticultural use throughout the United States and Japan.

This outbreak reconfirms sphagnum moss as a vehicle for sporotrichosis and suggests that workers in contact with the moss may reduce their risk of acquiring disease by wearing protective clothing. It also emphasizes the need for physicians to consider the possibility of sporotrichosis when patients present nonhealing ulcerating lesions on their extremities.

From *MMWR* 31:542, 1982.

Fig. 35-26. Tinea pedis. (Courtesy G. Roberts.)

may later appear. Chronic infections show peeling and cracking of the skin, often resembling eczema (Fig. 35-26). The causative agents of athlete's foot may be any number of species of *Microsporum, Trichophyton*, or *Epidermophyton*.

Tinea Barbae (Ringworm of the Beard Area)

Tinea barbae is a dermatophytic infection of the bearded areas of the face. Infection may remain superficial or may become severe and involve deeper tissue. Superficial infection appears as a scaly lesion; the deeper infection is characterized by deep pustules and nodular lesions (Fig. 35-27). Permanent loss of hair is common with the more severe infection. *T. rubrum* is the principal agent involved in superficial infections, while *T. verrucosum* is associated with the more severe form of the disease.

Tinea Favosa (Ringworm of the Scalp)

Tinea favosa is characterized by the formation of yellow, cup-shaped crusts of matted debris and mycelia on the scalp or torso. The crusts are called *scutula* (Fig. 35-28). The hair follicle is involved, and in more severe cases there is loss of hair. Most cases of disease are caused by *T. schoenleini*.

Fig. 35-27. Tinea barbae. Deep pustular folliculitis in patient with *Trichophyton verrucosum* infection. (From W. Rippon, *Medical Mycology* [2nd ed.]. Philadelphia: Saunders, 1982.)

Fig. 35-28. Tinea favosa. Seborrheic stage of disease showing matted hair and lesion with erythematous base. The infected hair is gray, whereas the normal hair is pigmented. (From W. Rippon, *Medical Mycology* [2nd ed.]. Philadelphia: Saunders, 1982.)

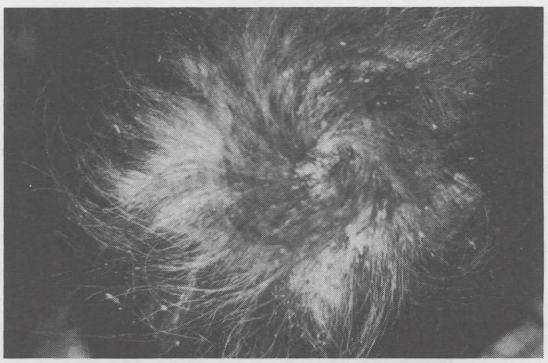
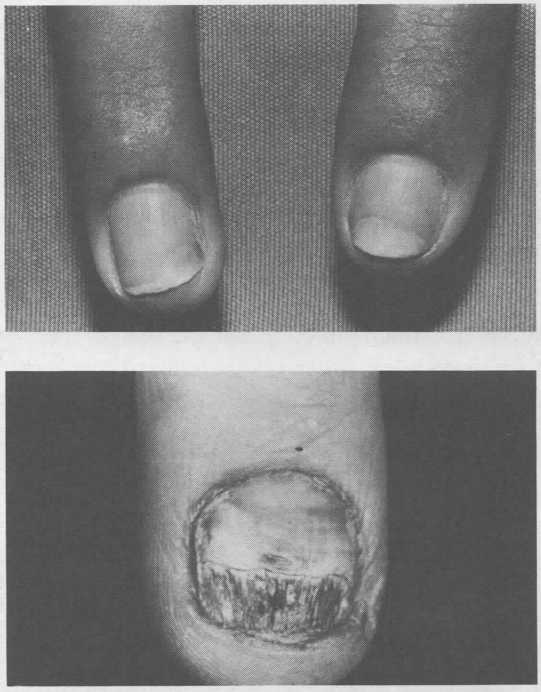


Fig. 35-29. Tinea unguium. A. Initial infection at distal edge of nail plate. B. Advanced disease showing grooved nails and dark brown coloration. (From W. Rippon, *Medical Mycology* [2nd ed.]. Philadelphia: Saunders, 1982.)



A

B

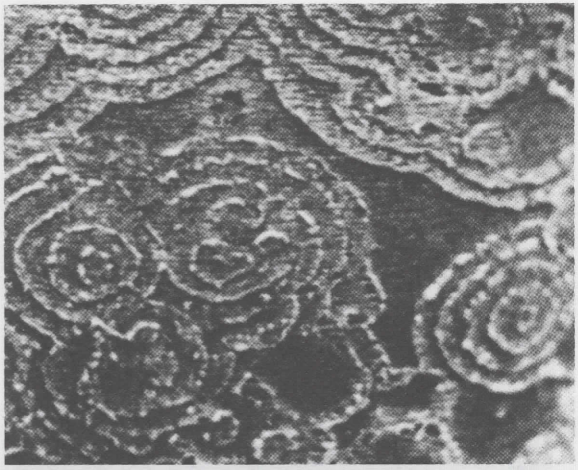
Tinea Unguium (Ringworm of the Nails)

Invasion of the nail plate is characteristic of tinea unguium. Infection may be of two types: (1) superficial involvement in which the fungus invades only the pits on the surface of the nail, and (2) involvement of the nail surface followed by invasion of the fungus beneath the nail plate (Fig. 35-29). Invasion of hyphae below the nail results in discoloration, keratinization, and distortion of the nail.

Tinea Imbricata (Ringworm of the Torso)

Tinea imbricata is restricted to certain areas of the world such as the Pacific Islands, Southeast Asia, and Central and South America. The infection is characterized by the formation of scaly, elevated concentric rings that may be scattered over most of the body (Fig. 35-30). The causal agent of disease is *T. concentricum*. The organism is believed to be transmitted by direct contact from mother to infant.

Fig. 35-30. Tinea imbricata produced by *Trichophyton concentricum* and manifested by concentric rings of scales. (From W. Rippon, *Medical Mycology* [2nd ed.]. Philadelphia: Saunders, 1982.)



Dermatophytes and Allergy

Many fungi act as allergens, causing allergic skin reactions. Allergic manifestations that appear in response to dermatophytosis and candidiasis are often referred to as *ids*. They appear as vesicular lesions at sites distant from the primary site of fungal infection and give rise to severe itching, especially after antifungal treatment.

SUPERFICIAL MYCOSES

Infections of the most superficial layers of skin and hair are termed *superficial mycoses*. These infections are generally innocuous and frequently reappear with or without treatment.

Tinea Versicolor

The causal agent of tinea versicolor is *Malassezia furfur*. The lesions, generally on the chest, back,

Fig. 35-31. Tinea versicolor. The pigmented areas represent the infected sites of the skin. (From W. Rippon, *Medical Mycology* [2nd ed.]. Philadelphia: Saunders, 1982.)

and shoulders, are tan and scaly (Fig. 35-31). There is little or no inflammation associated with them. Irradiation of the infected areas with Wood's light in the dark demonstrates orange-red fluorescing hyphae. Scales can also be removed from the infected areas and examined microscopically for the hyphal elements. A saturated solution of sodium thiosulfate applied two to three times daily is effective in treatment of superficial mycotic infections. Tolnaftate 1% in various creams and in polyethylene glycol also provides good results. Other drugs that have proved effective are selenium sulfide (1%) and salicylic acid (10–15%). Miconazole nitrate can also be used.

Tinea Nigra Palmaris

Tinea nigra palmaris is an asymptomatic infection that appears on the palms of the hands and fingers as dark brown or black macular areas. The condition is chronic and may last several years if left untreated. It is seldom seen in the United States. The causal agent is *Exophiala* (*Cladosporium*) *wernecki*. Scales removed from the skin should be examined microscopically on a wet mount containing 10% KOH. The wet mount is gently heated to break up keratinous cells, and this procedure allows one to see detached hyphae. The fungus appears in the form of highly branched hyphae. Treatment is the same as for tinea versicolor.

Black and White Piedra

Piedra is a fungus infection of the hair in the form of small nodules. *Black piedra* (caused by *Piedraia hortae*) affects the hair of the scalp, and the nodules produced are very dark. *White piedra* (caused by *Trichosporon beigelii*) affects the hairs of the beard, and the nodules produced are very light. The diseases are rarely seen in the United States. Infected hairs with attached nodules can be prepared in wet mounts containing 10% KOH. The nodules contain mycelia and can be examined microscopically. Treatment of black and white piedra is the same as for tinea versicolor.

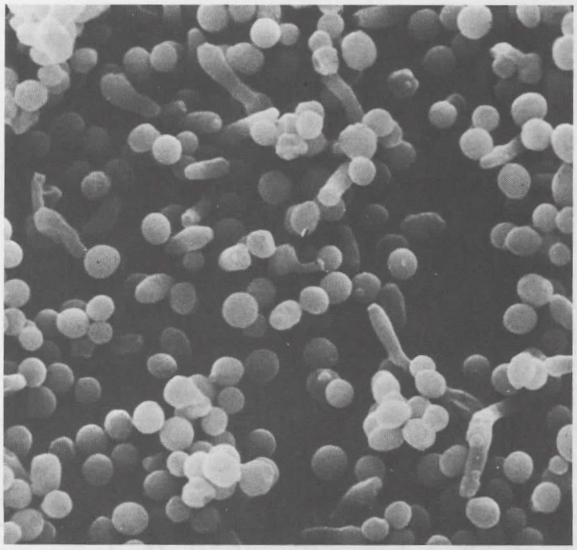
DISEASES CAUSED BY OPPORTUNISTIC FUNGAL PATHOGENS

In seriously debilitated and traumatized people, or those under treatment with broad-spectrum antibiotics and immunosuppressive drugs, nonpathogenic fungi may cause grave illness. These opportunistic fungi are found in the environment and belong to the following groups: *Candida, Aspergillus, Mucor*, and *Rhizopus*. These groups are the major opportunists, but remember that any of the fungi can also cause disease in the compromised host.

Candidiasis

GENERAL CHARACTERISTICS. *Candida* species are yeasts capable of producing pseudohyphae (Fig.

Fig. 35-32. Scanning electron micrographs of *Candida albicans*. A. Yeast form. B. Mycelial form. (From M. Borgers, *Rev. Infect. Dis.* 2:520, 1980.)



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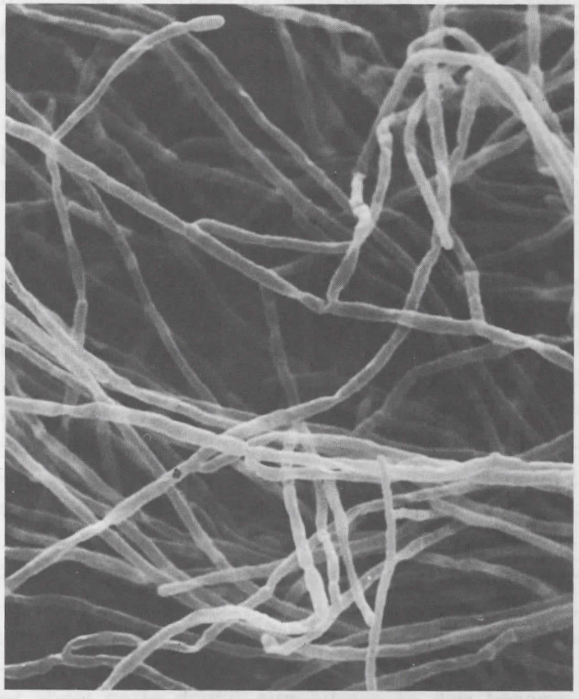

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35-32). They can be found as commensals in animals and humans, where they inhabit the respiratory tract, intestinal tract, skin, and female genital tract. Most infections are of the endogenous type (disease caused by one's own microbial flora), but transmission from mother to fetus and venereal transmission are also possible. The most important *Candida* species causing infection in humans is *C. albicans*, but other species such as *C. tropicalis, C. parapsilosis*, and *C. pseudotropicalis* are occasionally involved.

PATHOGENESIS. The frequency of *Candida* infections has risen dramatically since the advent of antibiotics and the increase in the number of surgical procedures, including transplants, in which antibiotics and immunosuppressive drugs are used. Infections are either cutaneous or disseminated and reflect the type of compromising condition that affects the host. Cutaneous diseases are usually naturally occurring and arise because of some condition such as diabetes or other endocrine imbalances, natural immunological deficiencies, or exposure of the skin to moist environments over long periods of time (e.g., in dishwashers and laundry workers). Disseminated infections are most often iatrogenic (produced by physicians; resulting from diagnostic procedures or treatment). The administration of some antibiotics and immunosuppressive drugs encourages the growth of fungal species such as *C. albicans* at the expense of bacterial species. The proliferation of *Candida* in the intestinal tract, for example, can lead to the hematogenous spread of the microorganism. The use of catheters, the implantation of prosthetic devices, and various types of surgery also provide an avenue for dissemination of *Candida*. *Candida* infections may be divided into three types: cutaneous, systemic or deep organ, and mucocutaneous.

Cutaneous Candidiasis. Oral *thrush* is one of the most common cutaneous candidal infections. In thrush the surface of the tongue is covered by white patches or pseudomembranes, which, if removed, reveal a raw, bleeding undersurface (Fig. 35-33). The pseudomembranes are composed of fungal cells, both yeast and hyphal forms, and epi-

Fig. 35-33. Thrush. Tongue of patient who died of leukemia and with secondary thrush. Note growth of *Candida* on surface of tongue. (From M. L. Dillon, Jr. In Y. Al-Doory [Ed.], *Epidemiology of Human Mycotic Disease*. Springfield, IL: Thomas, 1975.)

thelial debris. Thrush is most often observed in infants but can occur in any age group. Many infants appear to acquire the disease from mothers who have vaginal candidiasis. Endogenous thrush is a result of the administration of antibiotics, particularly broad-spectrum drugs, or various immunosuppressive agents that reduce the bacterial flora and permit *Candida* to divide unrestrictedly.

Vaginal candidiasis is frequently associated with diabetes, pregnancy, and antibacterial drugs. Lactobacilli in the vaginal tract are known to inhibit the growth of *Candida*. Women undergoing antibiotic therapy with drugs such as penicillin show reduced numbers of lactobacilli in the vagina. The reduction of these bacterial competitors permits the proliferation of *Candida*. The infection is characterized by inflammation of the vagina and the formation of a thick yellow-white discharge. Infection may spread to perianal areas and appear as a diaper rash in infants. Vaginal candidiasis may lead to candidiasis in a sexual partner after sexual intercourse. The condition in the male, called *candidal balanitis*, is characterized by superficial erosions and thin pustules on areas of the penis.

Individuals who have their appendages immersed in water for long periods of time or whose unexposed body sites are subject to a moist environment are also subject to infection by *Candida*

Fig. 35-34. Intertriginous candidiasis in a diabetic patient. Note "scalded skin" areas and satellite eruptions. (From W. Rippon, *Medical Mycology* [2nd ed.]. Philadelphia: Saunders, 1982.)

(*intertriginous candidiasis*). The feet, hands, groin, axillae, and intergluteal folds are the most commonly affected areas (Fig. 35-34). The lesions resemble eczema and may be scaly, creeping, and erythematous. Intertrigo may also be observed in diabetics, obese individuals, chronic alcoholics, or others with metabolic disorders.

Some of the most common cutaneous infections are those involving the nails (*onychia*) or around the nails (*paronychia*). These infections also result from immersion of appendages in water.

Systemic Candidiasis. Systemic candidiasis results from hematogenous spread of the microorganisms and may involve any organ or tissue of the body. The most critical sites of infection are the heart (pericarditis, myocarditis, endocarditis), spinal cord (meningitis), and urethra (urethritis, cystitis). More recently recognized conditions resulting from dissemination are arthritis and osteomyelitis. Heart infections may be the result of the use of contaminated needles by heroin addicts, surgery involving the implantation of prosthetic valves, and prolonged catheterization with polyethylene catheters. Neonates receiving hyperalimentation and broad-spectrum antibiotics are at an increased risk of candidemia which often leads to meningitis, arthritis, or osteomyelitis.

Chronic Mucocutaneous Candidiasis. Chronic mucocutaneous candidiasis (CMC) is associated with immunological deficiency. T cell immunodeficiency syndromes with failure in the cell-mediated immune response appear to be the initiating factor in disease. CMC is also frequently observed in patients with other underlying conditions that in some way affect the cell-mediated immune response. Patients are likely to lack delayed hypersensitivity to *Candida* and other skin test antigens. In addition, the lymphocytes of affected patients cannot be transformed in vitro by *Candida* antigen.

CMC infections involve the skin, mucous membranes, or any other epithelial surface including the respiratory tract, gastrointestinal tract, and genital epithelium. Most cases begin in infancy, but the disease may also involve those up to 30 years of age. The onset of disease seldom occurs after the age of 30. The disease is often refractory to treatment. Fatalities are rare, but when they occur are usually due to bacterial sepsis.

LABORATORY DIAGNOSIS. Presumptive identification of *Candida* species can be made by direct microscopical examination of clinical material for the presence of yeast cells or pseudohyphae (see Fig. 35-32). A valuable presumptive test is the germ tube test, in which a suspension of yeast cells from a colony is mixed with serum and incubated at 37°C for 2–4 hours. The mixture is then examined microscopically for the presence of *germ tubes*. Chlamydospore production in nutritionally deficient media such as rice extract agar and trypan blue agar is also used for identification of the fungus. If chlamydospores are not formed, it may be necessary to determine the sugar fermentation and sugar assimilation pattern to distinguish between *C. albicans* and other *Candida* species. *Candida* is the only fungus to produce both yeast cells and pseudohyphae in tissue. Blastospores and pseudohyphae can be demonstrated in biopsy tissue by staining techniques. For systemic *Candida* infections, the latex agglutination test has proved to be an invaluable diagnostic as well as prognostic tool.

TREATMENT. Most cutaneous infections are treated with creams or powders containing nystatin, miconazole, or gentian violet. Amphotericin B has been used in severe systemic mycoses, but its therapeutic dose level is close to the toxic dose level and it must therefore be administered with caution. Amphotericin B is toxic to the kidneys and should not be given to persons suffering from systemic candidiasis after kidney transplantation. The new drug flucytosine has been employed as a replacement for amphotericin B; however, some cases of fatal aplastic anemia have been reported when flucytosine was used. Its administration should be correlated with periodic patient hemograms during the course of therapy. Over 50 percent of clinical isolates of *C. albicans* develop resistance to flucytosine. Treatment of mucocutaneous candidiasis with antifungal agents alone has not met with success, and relapses are frequent. Bone marrow transplantation combined with amphotericin B therapy has been successful in a few special cases. A more recent development involves the administration of Lawrence transfer factor derived from individuals with specific delayed hypersensitivity. Transfer factor is a component obtained from lymphocytes sensitized with specific antigens. It can apparently transfer cell-mediated immunity to a nonreactive individual in some cases but has not been universally successful as yet.

Aspergillosis

Aspergillosis represents a spectrum of diseases caused by species of *Aspergillus*. These organisms are ubiquitous and are common to the soil, water, decaying vegetation, and any area that contains organic debris. The respiratory tract is the principal portal of entry, and inhaled spores can cause disease in healthy as well as compromised patients. The species most frequently associated with disease is *A. fumigatus*, but other species such as *A. flavus, A. niger*, and *A. terreus* are occasionally involved.

PATHOGENESIS. The clinical manifestations of aspergillosis can be divided into three types: allergic, colonizing (aspergilloma), and invasive.

Allergic aspergillosis is an asthma-like response

to an allergen, which in this instance is the fungal spore. The spores usually fail to germinate unless the host is severely compromised. This asthma-like illness is characterized by fever, cough, and wheezing induced by the presence of reaginic IgE antibodies. The syndrome may become severe and chronic with bronchial plugging. Occasionally in the chronic form the spores will germinate and be released from fruiting heads (see Laboratory Diagnosis).

Colonizing aspergillosis, which may develop after allergic aspergillosis, is characterized by the formation of a fungus ball (aspergilloma), a dense collection of hyphae. The fungus grows out of and colonizes the pulmonary surfaces. Symptoms are similar to those of allergic aspergillosis, but spitting of blood (hemoptysis) is frequently encountered.

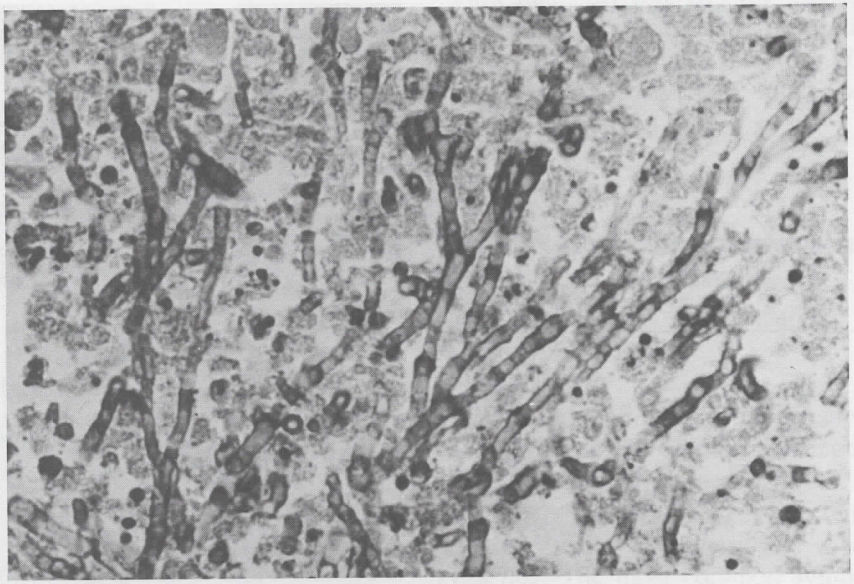
Invasive aspergillosis can develop from the allergic or colonizing forms of the disease, or it can arise independently. *Invasive* implies that *Aspergillus* hyphae have penetrated tissue either as a localized or disseminated form of the disease. Several factors may predispose the patient to invasive disease: (1) cytotoxic chemotherapy, (2) therapy with broad-spectrum antimicrobial agents, (3) leukopenia, and (4) acute leukemia. Mortality is very high, especially among renal transplant patients and leukemia or lymphoma patients. Invasive disease affects primarily the respiratory tract. Usually the fungus colonizes the tracheobronchial tree, and this leads to bronchopneumonia. Hyphae invade the lumina and walls of blood vessels causing pulmonary hemorrhage. Lung tissue may also be invaded as a direct result of hematogenous spread, for example, after parenteral therapy. Untreated invasive disease results in death within 2 weeks of onset of symptoms. Invasive disease may also affect the nose and paranasal sinuses. This type of infection is endemic in the Sudan. Infections have also involved the ear canal, the eye, and the heart. Endocarditis caused by aspergilli is associated with prosthetic valve implantation.

Disseminated aspergillosis following invasive disease is the most severe form and invariably results in death. Either antimicrobial agents or glucosteroids have been implicated as predisposing factors, but occasionally even healthy individuals may develop disseminated disease. There is widespread organ involvement, with the lung as the principal target. Death usually results from bilateral pneumonia or intracerebral hemorrhage with fungal invasion and thrombosis.

Considerable research with murine and human cells in vitro has led to some important discoveries concerning the pathogenesis of aspergillosis. It appears that two lines of host defense must be breached before aspergilli can invade tissue. Neutrophils are the principal line of defense against the mycelial form of *Aspergillus*, while macrophages (monocytes) are efficient in eradicating spores. Even in humans with chronic granulomatous disease *Aspergillus* infection is not as fulminant as might be expected. It is believed that the ability of monocytes to damage hyphae provides some level of protection in these compromised individuals. The host's first line of defense is the macrophage, which kills conidia; the second line of defense is the neutrophil, which kills hyphae. Investigations of compromised patients have also revealed that those who receive corticosteroids are at more risk from invasive aspergillosis than patients receiving other drugs or antibiotics. Corticosteroids have been shown to impair the ability of macrophages to inhibit germination of ingested conidia. Corticosteroids are known to produce a cumulative response. When renal transplant centers reduced the dosage of corticosteroids the frequency of fungal infections also decreased precipitously.

LABORATORY DIAGNOSIS. Diagnosis of aspergillosis is dependent on demonstrating fungal elements in pathologic material and repeated isolation of *Aspergillus* from clinical specimens. Identification in tissue is dependent on (1) finding uniform-sized hyphae 3 to 4 μm in width (Fig. 35-35), (2) finding characteristic dichotomous branching of hyphae at 45-degree angles, (3) the staining of hyphae by special stains, (4) a positive fluorescent antibody response, (5) the presence of characteristic conidiophores (Fig. 35-36), and (6) the presence of septate hyphae. Tissue identification techniques should also be backed up by cultural tests since it may not

Fig. 35-35. *Aspergillus* hyphae in lung tissue. (Courtesy K. J. Kwon-Chung.)



be possible to evaluate all the criteria in tissue. In addition some of these morphological characteristics are also found with other fungi. Because of the widespread occurrence of aspergilli in the environment, a positive culture of *Aspergillus* from human tissue must be carefully evaluated. Invasive disease is difficult to diagnose without biopsy, a procedure that many patients cannot withstand. Serological techniques therefore play an important role in diagnosis; however, in immunocompromised patients these tests are of limited value because antibody elaboration is minimal. An early, accurate diagnosis of invasive aspergillosis can be made by monitoring the patient using immunodiffusion, counterimmunoelectrophoresis, or other tests for specific precipitins to *Aspergillus* antigens.

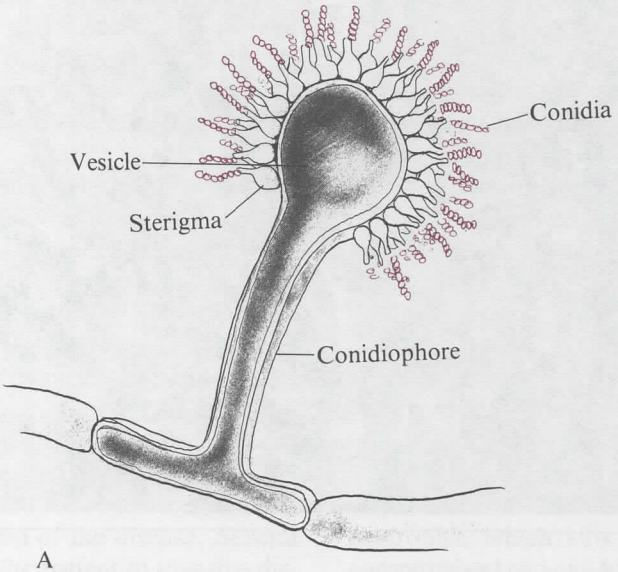
TREATMENT. Invasive aspergillosis is a highly fatal disease, and prompt therapy is required. The mortality rate for those with leukemia is approximately 70 percent but only 25 percent for those with pulmonary disease following transplantation. Amphotericin B alone or in combination with flucytosine has been used with some success in invasive disease. Allergic forms of disease can be treated with corticosteroids (but not in immunosuppressed patients). Surgical resection may be necessary for aspergilloma.

Mucormycosis

Mucormycosis (also called *phycomycosis* or *zygomycosis*) refers to diseases caused by members of the order Mucorales. The causal agents of disease include species of *Rhizopus, Absidia, Mucor, Saksenaea, Cunninghamella*, and others. The members of these genera are inhabitants of the soil and are commonly found on fruits and bread. Infections occur in compromised patients such as those with ketoacidosis resulting from diabetes but also in leukemic patients, burn patients, and those undergoing immunosuppressive therapy.

PATHOGENESIS. The most severe forms of mucormycosis involve the nasal region (rhinocerebral mucormycosis) and lung (thoracic mucormycosis). Sporangiospores of the causal agent are inhaled

Fig. 35-36. *Aspergillus* conidiophore. A. Diagram. B. Scanning electron micrograph of the conidiophore, terminal vesicle, sterigma, and conidia of *Aspergillus flavus*. (From E. U. King and M. F. Brown, *Can. J. Microbiol.* 29:653, 1983.)



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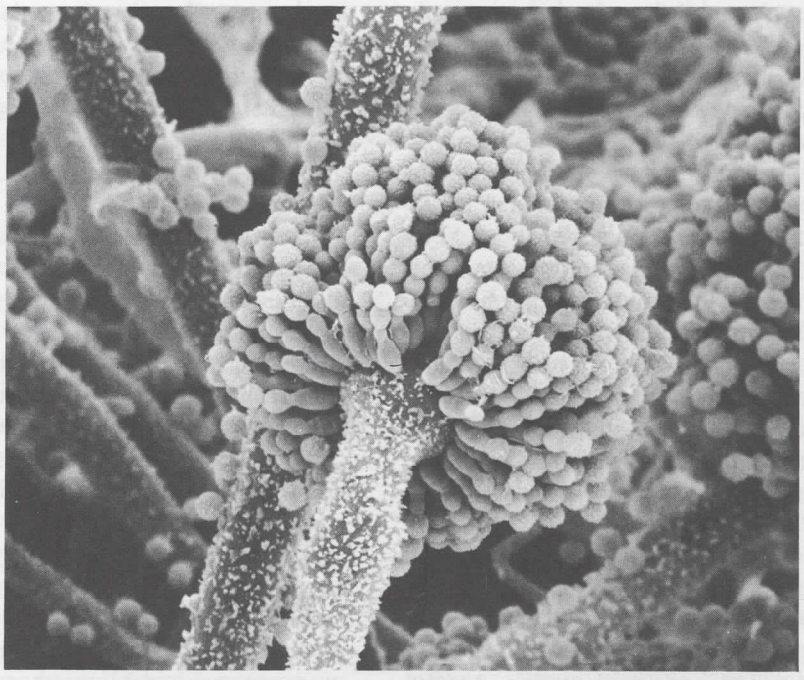

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Fig. 35-37. Mucormycosis. (Courtesy G. Roberts.)

and germinate either in the nasal surfaces or in the lung. Nasal invasion takes place with the formation of hyphae that can spread and involve the orbit, face, meninges, and frontal lobes of the brain. Edema and necrotic tissue are found around the eye and in the nose (Fig. 35-37). Death can occur within a few days of onset.

Hyphae that invade the lung cause mass lesions and bronchopneumonia. Hyphae invade the walls of blood vessels and cause thrombosis. Dissemination of disease to the central nervous system is a frequent complication of pulmonary infection.

LABORATORY DIAGNOSIS. Diagnosis is usually made by the observation of broad (10- to 15-μm) aseptate hyphae in normally sterile body fluids or tissue scrapings or biopsy specimens (Fig. 35-38). Since *Rhizopus* is a common contaminant in the laboratory, recovery of this organism from culture is not diagnostic unless accompanied by clinical signs in the patient.

TREATMENT. The mortality rate from invasive mucormycosis is high (approximately 90 percent). Viral and bacterial infections are frequently observed concomitantly with fungal disease and may be the cause of many fatalities. If recognized in time mucormycosis can be treated with amphotericin B administered IV.

MYCOTOXINS (MYCETISMUS AND MYCOTOXICOSIS)

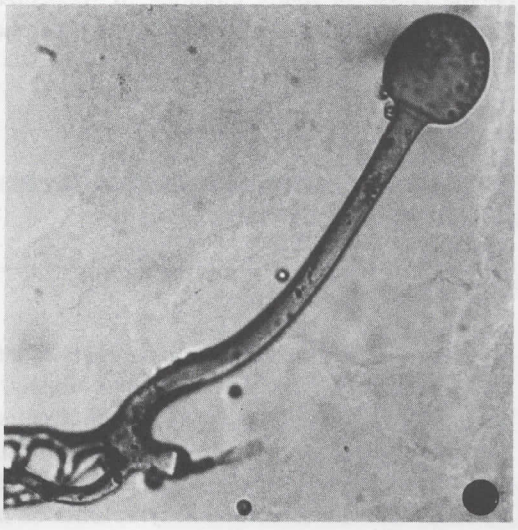
MYCETISMUS

Mycetismus, or mushroom poisoning, is caused by the ingestion of certain poisonous fleshy field fungi. Mushroom poisoning is not as common in the United States as it is in areas of eastern Europe where mushroom gathering is a common family practice. The most common symptoms of mushroom poisoning involve the gastrointestinal tract and nervous system. Symptoms, which appear 1–2 hours after ingestion, include profuse sweating, violent gastrointestinal involvement, and convulsions. If the dose of toxin is sufficiently high, death may occur within 10–12 hours of onset. The most toxic mushrooms are those belonging to the genus *Amanita*.

MYCOTOXICOSIS

Unlike mycetismus, in which the toxic substance is part of the fungal tissue, mycotoxicosis is due to a toxin that is produced as a secondary metabolite. The mycotoxins of interest are produced by species of the genera *Penicillium, Fusarium, Streptomyces*, and *Aspergillus*. These organisms are a common contaminant of grains and foods. Mycotoxins are intimately associated with every food or feed and can be produced at every stage of harvesting, production, and storage of food. When certain environmental conditions prevail (moisture content, temperature, and others) the fungal contaminants grow and release toxic secondary metabo-

Fig. 35-38. *Rhizopus rhizopodoformis* isolated from diabetic patient. A. Sporangiophore (×250). B. Sporangiospores (×500). C. Stain of hyphae in abscess (×1000). (From B. C. West et al., *J. Clin. Microbiol.* 18:1384, 1983.)



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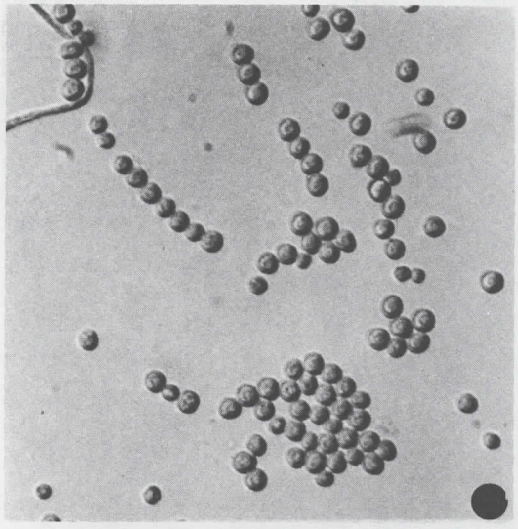

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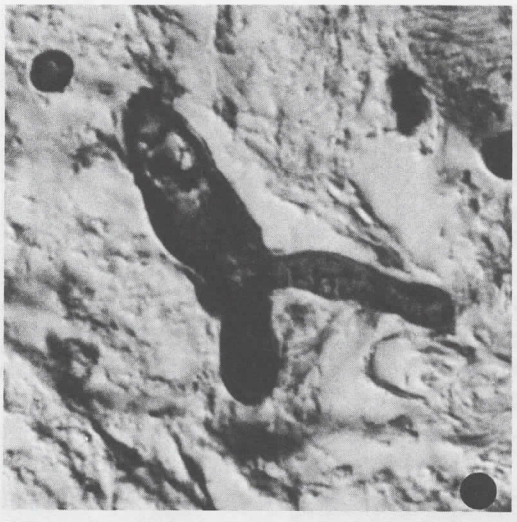

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lites. One of the more widely recognized mycotoxins is *aflatoxin*, which is produced by *Aspergillus flavus*. Aflatoxin was first discovered in 1960 when 100,000 turkeys and several thousand other domestic birds died after ingesting contaminated Brazilian peanut meal. Investigations into the cause of death revealed that the bird feed was contaminated by *A. flavus*.

The animal population displays a wide range of susceptibility to aflatoxin. Young pigs and calves are more vulnerable to the action of the toxin than are other barnyard animals. Low levels of aflatoxin stunt their growth and increase their susceptibility to infectious disease. Aflatoxin is a potent carcinogen in animals, and in humans it is believed to be responsible for cancer of the liver. Studies show that liver cancers occur with greater frequency in countries or areas where aflatoxin is known to grossly contaminate stored grains. In parts of the Far East and India, maize, corn, rice, and other grains that have high concentrations of aflatoxin are consumed in large amounts. Autopsies on individuals there reveal that many organs contain high levels of aflatoxin.

The effects of aflatoxin on human metabolism have not been fully elucidated. Animal studies reveal, however, that lipid and vitamin metabolism are severely affected. During aflatoxicosis in chickens, fatty liver and steatorrhea (increased lipid in the excreta) appear to be associated with a decrease in bile salts and the main fat-digesting enzyme, pancreatic lipase. The effects on lipid metabolism from aflatoxin can be reduced if animals are given high concentrations of fat in their diet. This result is believed to explain the varied response of individuals to apparently similar doses of aflatoxin in their diet. Aflatoxin also interacts with certain vitamins. Experiments in chickens and cattle indicate that aflatoxin reduces the vitamin A, vitamin D, and riboflavin content of vital organs and tissue. A natural or induced deficiency of these vitamins makes the animals more susceptible to the effects of aflatoxin. A deficiency of thiamine, however, exerts a protective effect against aflatoxicosis in chickens, probably because thiamine deficiency stimulates lipid metabolism, which is normally inhibited by aflatoxin.

Mycotoxins of current concern are produced by species of *Fusarium*. Two of these toxins are called *T-2* and *vomitoxin*. Like aflatoxin these toxins are also produced by growth of the fungal species on various grains and have been implicated in animal disease. *Fusarium*-infected feed has caused disease in humans in countries outside the United States. There have been reports from Southeast Asia that *Fusarium* toxins have been used in chemical warfare. The term *yellow rain* has been used because of the color of these toxic materials supposedly released from aircraft. Contact with the toxin causes vomiting, skin irritations, bleeding, and death. Some scientific agencies, however, identify the cause of the problem as a natural occurrence of the toxins resulting from prior defoliation or the use of napalm.

SUMMARY

Fungi are eukaryotic cells that can be morphologically divided into the spherical yeasts and the filamentous molds. Yeasts reproduce primarily by budding, while molds reproduce by asexual or sexual spore formation. Spores may be produced on specialized hyphal structures, or they may be produced directly from hyphae. Sabouraud medium, the most common medium for isolation of fungi, is at a pH that inhibits bacterial growth.

Fungal diseases are acquired primarily by inhalation of fungal spores or by contact of spores with tissue or mucous membranes in abrasions. Most fungal diseases occur in compromised individuals. Immunity to fungal infection is primarily by the cell-mediated response. Humoral immunity appears to play only a minor role in protecting the host. The lack of specific antibodies makes serodiagnosis of many fungal diseases difficult. Dimorphism plays an important role in the pathogenesis of many fungal agents. Fungal diseases can be broken down into five types: systemic, subcutaneous, cutaneous, superficial, and opportunistic.

Systemic diseases are acquired by inhalation of spores. The initial infection is pulmonary, and this usually subsides as the pathogen is disseminated in the body. The systemic diseases include histoplasmosis (caused by *Histoplasma capsulatum*), coccidioidomycosis (*Coccidioides immitis*), blastomycosis (*Blastomyces dermatitidis*), paracoccidioidomycosis (*Paracoccidioides brasiliensis*), and cryptococcosis (*Cryptococcus neoformans*).

Subcutaneous mycoses are usually acquired via puncture wounds while working with soil or its associated environment. These fungal agents cannot invade the deeper tissue, and their lesions are easily observed on the skin or in the lymphatics. The most frequently observed subcutaneous mycosis in the United States is sporotrichosis.

Cutaneous fungal infections are caused by dermatophytes that invade keratinized areas of the body. The principal genera infective for humans are *Trichophyton, Microsporum*, and *Epidermophyton*. Some infections can be acquired by contact with clothing or articles contaminated by human dermatophytes. Other cutaneous infections can be acquired from animals who carry the infectious fungal agent. The infections are referred to as ringworm or tineas.

Superficial mycoses involve superficial areas of the skin and usually do not require treatment. Lesions are usually scaly eruptions on the chest, back, and shoulders. Some pathogens can attack the hair on the scalp or the beard.

Opportunistic fungal pathogens cause disease primarily in (1) compromised patients receiving antimicrobial therapy, (2) those receiving immunsuppressive drugs, and (3) those with metabolic disorders. The principal agents of disease are *Candida* (especially *C. albicans*) and *Aspergillus* (*A. fumigatus*), while *Mucor* and *Rhizopus* are only occasionally involved. Only *Candida* is part of the normal microbial flora of humans. It causes three types of infections: cutaneous, systemic, and mucocutaneous. Systemic disease can be fatal. Aspergillosis is caused primarily by *A. fumigatus*. There are three types: allergic, colonizing, and invasive. Invasive disease, which almost invariably results in death if left untreated, occurs in those patients in whom neutrophils and macrophages have been rendered useless. *Rhizopus* and *Mucor* species cause infections primarily in those with metabolic disorders such as diabetes. Mortality from the diseases caused by these species is very high.

Fungi can be toxic because of the nature of their own tissue (mushrooms) or because they produce secondary metabolites when growing on various foods or grains (mycotoxicosis). One of the most toxic secondary metabolites is aflatoxin, which is believed to be a cause of liver cancer when contaminated grains are ingested over long periods of time.

QUESTIONS FOR STUDY

1. What is meant by *dimorphism*? What is its relationship to pathogenesis among certain fungi?
2. Describe the asexual spores that arise directly from hyphae. Describe the spores that arise from specialized hyphal structures.
3. What types of media are used in the isolation of fungi?
4. Why are systemic mycoses so difficult to diagnose?
5. Which fungal diseases are contagious?
6. What are the most important antifungal agents used in the treatment of systemic mycoses?
7. What is the difference between mycetismus and mycotoxicosis?
8. What fungal species is (are) found in soils containing high levels of avian feces?
9. Are serological techniques effective in the diagnosis of fungal disease? Explain.

10. Which systemic fungal disease is acquired in hot, arid climates and primarily by dark-skinned individuals?
11. Are any special precautions necessary in the laboratory diagnosis of fungal disease?
12. Which of the agents of systemic mycoses is not dimorphic? What is the pathogenic mechanism for this species?
13. What is the most common subcutaneous mycosis in the United States?
14. How are most subcutaneous mycoses acquired?
15. What are dermatophytes, and where can they be found? What are the three major genera of dermatophytes infective for humans?
16. What are tineas?
17. What is the technique for preparing a slide containing a fungal-contaminated skin specimen?
18. What are the three clinical manifestations of *Aspergillus* infection?
19. Which fungal species is considered the most common opportunistic pathogen? Where is it found? What are some of the clinical manifestations of infection by this microorganism?
20. What is the host's line of defense against infections by *Aspergillus* species? What are some of the reasons for invasive *Aspergillus* infections?

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VIII. MEDICAL PARASITOLOGY

36. THE PROTOZOA, HELMINTHS, AND ARTHROPODS

OBJECTIVES

To discuss parasitism and parasitic disease in terms of types of life cycles and hosts, adaptations, modes of transmission, factors that affect the geographic distribution and incidence, laboratory identification procedures, and control and treatment measures

To give the name of the parasite, the principal clinical manifestations, identification procedures, mode of transmission, and treatment and control measures of the various parasitic diseases

To associate with specific parasites: pan amebic meningoencephalitis, opportunistic pathogens in the immunodepressed, visceral and larval migrans, pinworm or seatworm, muscle worm, swimmer's itch; improperly cooked pork, cats, beavers, female anopheles mosquito

To comment on the importance of the changing of surface antigens by trypanosomes

To summarize the disease-related involvements of invertebrate arthropods

- General Characteristics of Parasites and Parasitic Infections
  - Incidence of Parasitic Infection
    - Parasitic Infection in the United States
  - Survival of Parasitic Species
    - Adaptations
  - Infective Forms and Life Cycles of Parasites
    - Representative Life Cycles
  - Factors That Influence the Incidence of Parasitic Infection
  - The Nature of Parasitic Disease
  - Diagnosis of Parasitic Infection
    - Direct Examination
    - Identification of Intestinal Parasites
    - Blood Smears
    - Tissue Examination
    - Unique Techniques
    - Serology of Parasitic Infection
  - Control and Treatment of Parasitic Infection
    - Immunization
    - Chemotherapy
- The Protozoa
  - Sarcodina—the Amebae
  - Mastigophora—the Flagellated Protozoa
  - Ciliata or Ciliophora—the Ciliated Protozoa
  - Sporozoa
  - Protozoal Diseases Caused by the Amebae
    - Amebiasis and Amebic Dysentery
      - Symptoms and Pathogenesis
        - Intestinal Amebiasis
        - Extraintestinal Amebiasis
      - Laboratory Diagnosis
      - Treatment and Control
  - *Dientamoeba fragilis*
  - Primary Amebic Meningoencephalitis
  - Granulomatous Amebic Meningoencephalitis and Disseminated Acanthamebiasis
- Protozoal Diseases Caused by the Flagellates
  - Giardiasis (Lambliasis)
    - Symptoms and pathogenesis
    - Epidemiology
    - Laboratory Diagnosis
    - Treatment
  - Trichomoniasis
    - *Trichomonas tenax* and *Trichomonas hominis*
    - *Trichomonas vaginalis*
      - Infections in Women
      - Infections in Men
      - Diagnosis
      - Treatment
  - Trypanosomiasis
    - African Trypanosomiasis (African Sleeping Sickness)
      - Continual Variation of Surface Antigens as a Means of Evading Existing Antibody
      - Laboratory Diagnosis
      - Treatment and Control
    - American Trypanosomiasis (Chagas' Disease)
  - Leishmaniasis
    - Visceral Leishmaniasis (Kala-azar)
    - Cutaneous Leishmaniasis
    - Mucocutaneous Leishmaniasis
- Protozoal Diseases Caused by the Ciliates
  - Balantidiasis (Ciliary Dysentery)
- Protozoal Diseases Caused by the Sporozoa
  - Malaria
    - Life Cycle
    - Symptoms and Pathogenesis
    - Laboratory Diagnosis
    - Treatment and Control
  - Toxoplasmosis
    - *Toxoplasma gondii* in Members of the Cat Family
    - *Toxoplasma gondii* Infections in Humans
    - Laboratory Diagnosis
    - Treatment and Control
  - Babesiosis (Nantucket Fever)
  - Pneumocystosis
  - Cryptosporidium Species—Cryptosporidiosis
- The Nemathelminthes
  - Ascariasis (Roundworm Infection)
    - Symptoms and Pathogenesis
    - Laboratory Diagnosis
    - Treatment
    - Visceral Larva Migrans
  - Hookworm Infection
    - Laboratory Diagnosis
    - Treatment and Control
    - Cutaneous Larva Migrans

Strongyloidiasis
Enterobiasis (Pinworm or Seatworm Infection)
Symptoms
Laboratory Diagnosis
Treatment and Control
Trichinosis (Muscle Worm Infection)
Life Cycle
Symptoms and Pathology
Laboratory Diagnosis
Treatment and Control
Filariasis
Symptoms and Pathogenesis
Laboratory Diagnosis
Treatment
The Platyhelminthes
Trematoda—Flukes
Schistosomiasis (Bilharziasis)
Life Cycle
Pathogenesis
Geographical Distribution
Laboratory Diagnosis
Treatment
Schistosome Dermatitis
Cestoidea—Tapeworms
Beef Tapeworm and Pork Tapeworm Infection
Diphyllobothriasis
The Arthropoda

GENERAL CHARACTERISTICS OF PARASITES AND PARASITIC INFECTIONS

Medical parasitology deals with those members of the animal kingdom that sometime in their life cycle take up temporary or permanent abode in or on a human host. It includes consideration of arthropods that cause discomfort and sometimes death by the injection of venoms. Disease may occur during the period when the parasite is dependent on the human host for shelter and sustenance; this is the ecological niche where a part of its life cycle is spent. Other medically important infectious agents cause disease largely under the circumstances of a parasitic relationship, but, traditionally, the terms *the parasites* or *parasites* refer to organisms from the animal kingdom that cause disease.

Parasites are found in four divisions of the animal kingdom: the Protozoa, the single-celled animals; the Nemathelminthes, the roundworms; the Platyhelminthes, the flatworms; and the Arthropoda, invertebrate animals with jointed appendages. Most of the adult forms of the latter three phyla are macroscopic in size. It may seem incongruous that microbiology, which by definition is the study of microscopic forms, includes the study of organisms that are readily observed by the unaided eye. *Ascaris* worms are the size of earthworms. The large tapeworms attain a length of 25 to 30 feet. Medical microbiology encompasses the study not only of disease-causing microscopic forms but of all infectious agents, the macroscopic parasites included. The microscope does figure prominently in the identification of the ova and larvae of the macroscopic organisms.

INCIDENCE OF PARASITIC INFECTION

Industrialized nations are not highly cognizant of the incidence of parasitic infections and how they affect the quality of the lives of a large segment of the human population. Millions of people, particularly in the tropics and subtropics, are debilitated, disfigured, blinded, and inconvenienced by such infections. Many are diseases of the poor, the lower socioeconomic groups, those in primitive surroundings, and those in substandard living conditions. The disquieting statistics include the following facts: One in four people in the world suffers malaria or is exposed to it. One in five individuals suffers from hookworm infection, with energies depleted by the bloodsucking worms attached to the intestinal wall. Fifty million Africans harbor trypanosomes, which cause an infection that occasionally develops into fatal sleeping sickness. One of every four inhabitants of earth carries round-

worms in the intestinal tract. Similar figures of the incidence of infection can be quoted for many of the parasites.

Parasitic Infection in the United States

Parasitic infections in the United States are common enough, but morbidity and mortality are very low. The better-known parasitic diseases are due to the protozoal infections of amebiasis, giardiasis, vaginal trichomoniasis, and toxoplasmosis and the helminthic infections of enterobiasis and trichinelliasis. Congenital toxoplasmosis seems to be the most serious of the indigenous infections on account of the occurrence of mental retardation and visual impairment or loss. The popularity of travel and the influx of immigrants account for the appearance of sporadic cases of exotic parasitic infections in this country.

SURVIVAL OF PARASITIC SPECIES

Certain requirements need to be fulfilled for parasitic infection to occur and on a broader scale for the parasitic species to survive. The parasite must gain entrance to the host, survive and reproduce there, eventually leave the host, and chance upon suitable environmental conditions (e.g., moisture, temperature) or contact other appropriate hosts and vectors. For many parasites other appropriate hosts are intermediate hosts where a developmental stage (e.g., the larval form of worms) in the life of the parasite occurs. It is in the requirement for specific intermediate hosts that a number of parasites differ from the other infectious agents for transmission and for survival of species.

Adaptations

The ability of the parasite to multiply and survive in the host is related to adaptations undergone by the species in its evolution. The gain or loss of structures and markedly increased reproductive capacities are examples of differences between parasites and their free-living counterparts. Tapeworms lack digestive tracts. They need only absorb nutrients from the host's digestive tract. Various species of parasites have holdfast structures such as hooks, suckers, and suctorial grooves by which they become embedded in or attached to the host tissues. The movement of food and the contractile waves of the host intestine therefore do not dislodge the anchored parasite.

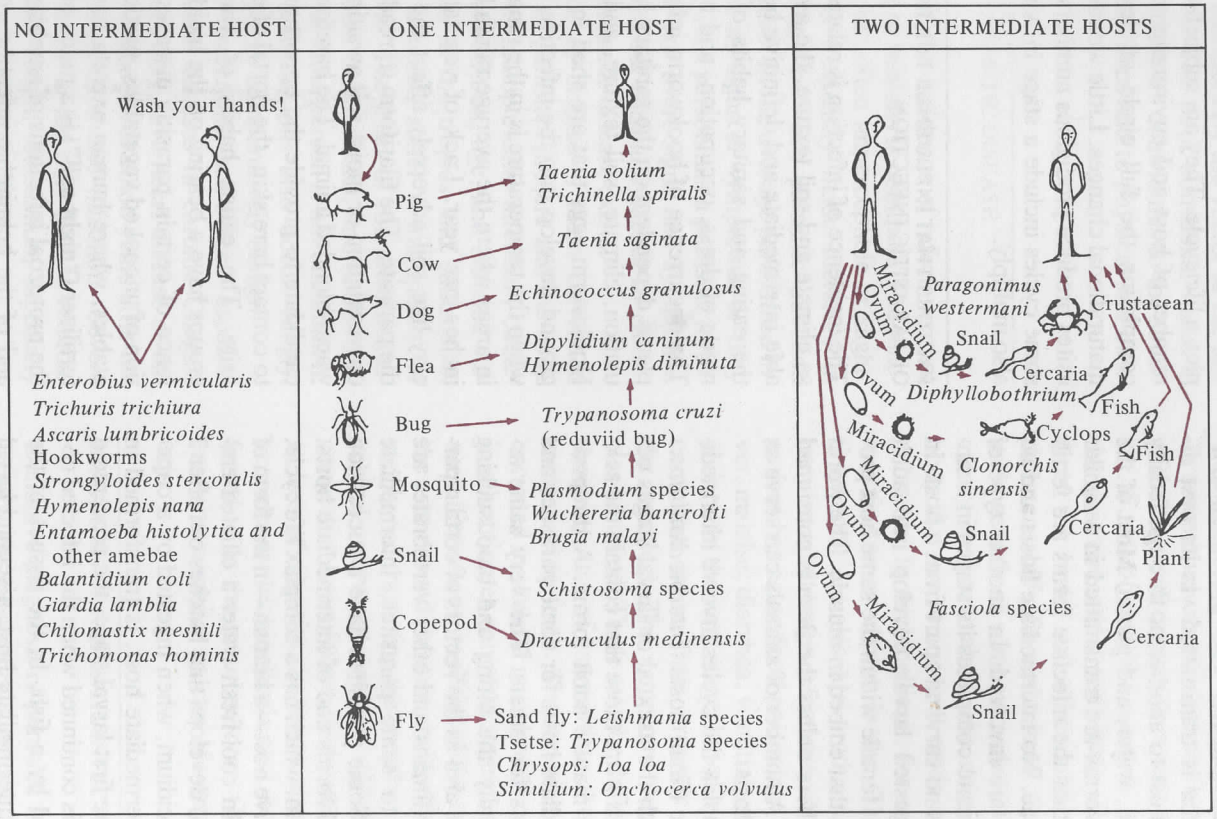
The really noteworthy specializations are in the increased reproductive capacity of certain parasites and in the complex life cycles some have. The adult female *Ascaris lumbricoides* worm produces approximately 200,000 eggs per day. In addition to a striking egg-producing capacity, some species multiply in their larval forms in intermediate hosts. A single schistosome egg, for example, hatches into a ciliated miracidium. The miracidium in its specific species of snail develops into hundreds to thousands of cercariae, the larval forms that infect humans and animals.

INFECTIVE FORMS AND LIFE CYCLES OF PARASITES

Most parasites undergo a series of developmental stages during which they grow, mature, and reproduce. They exist in more than one form. Many of the protozoa exist not only in the active, feeding, proliferative form, the *trophozoite*, but also in a resistant, quiescent form, the *cyst*. The trophozoite causes the disease; the cyst serves as the infective, transmitted form. Most of the worms produce ova (are oviparous) from which embryos or larval forms develop. Others directly produce live larvae (are viviparous). The fertilized ova or the larvae are the infective forms from which the adults develop. The adult worms are usually responsible for the symptoms and pathological conditions of the disease. The ova, as in schistosomiasis, and the larvae, as in trichinelliasis, however, may cause the pathology and the symptoms.

Some parasites spend their lives in the same host, generation after generation. Some leave the host and remain quiescent in nature until they enter another host. Others undergo active growth and metamorphosis outside of the final host. In some there are sexual and asexual forms. In some the parasite is able to develop in more than one host (Fig. 36-1).

Fig. 36-1. Overview of the life cycles of parasites of importance to humans. (From E. W. Koneman et al., *Color Atlas and Textbook of Diagnostic Microbiology*. Philadelphia: Lippincott, 1979.)



Representative Life Cycles

Examples of parasitic life cycles reflect their diversity and their complexity. The protozoon *Entamoeba histolytica* is transmitted via its cyst directly from one host to another or through fecally contaminated soil, water, and plants. Most of the intestinal roundworms are transmitted in a similar manner, except that the infective forms are fertilized eggs or larvae. No intermediate hosts and no alternative hosts are involved. In another type of life cycle the parasite completes its stages in a single host. There is no survival apart from a host. In trichinelliasis ingested larvae develop into adult worms. The adult female within the same host produces live larvae that embed in muscle. The worm is at a dead end here unless the flesh is consumed by another host. A number of animals can serve as hosts in trichinelliasis.

The more complex life cycles involve intermediate and definitive (final) hosts. *Intermediate hosts* are those in which the asexual or larval stages occur. *Definitive hosts* are those that contain the sexual forms or the mature adult forms. Arthropods serve as intermediate hosts for some parasites and as definitive hosts for others. The very same arthropods, especially the biting and bloodsucking arthropods, serve also as the vectors of certain parasitic diseases. Humans and other vertebrates are definitive hosts to some parasites, intermediate hosts to others. Some parasites have a succession of developmental forms and of intermediate hosts. The fish tapeworm, which has a complex life cycle, leaves the definitive host—a human—in the form of a fertilized egg. In cool fresh water a ciliated embryo (coracidium) develops that hatches out after 2 weeks. The coracidium, when ingested by a copepod, the first intermediate host, is transformed in 2–3 weeks into the first larval stage, the procercoid larva. The cycle is continued when the infected copepod is ingested by a fish. In the tissues of the fish, the second intermediate host, a second larval stage, the pleroceroid larva, forms. Consumption of raw or improperly cooked pleroceroid-containing fish by a human or another animal completes the cycle. The adult tapeworm develops, and the eggs shed in the feces into fresh water initiate another cycle.

It is evident that continuation of the species is at an even greater risk for parasites that have so complex a life cycle. They are subjected to an increased number of host and environmental changes while completing the full circle of developmental and maturational changes. Little wonder that some parasites produce prodigious numbers of eggs and that some cycles include a stage in which larval forms also multiply.

FACTORS THAT INFLUENCE THE INCIDENCE OF PARASITIC INFECTION

The incidence of infection is related to such factors as climate and soil texture, the availability of suitable intermediate and definitive hosts and vectors, the eating and sanitary habits of the population, mode of dress, occupation, and nutritional status. The occurrence of hookworm infection, for example, is dependent on the sanitary habits of the population, climate, soil texture, and mode of dress. Hookworm eggs that are shed in the feces on the ground develop into the infective larval form only when the temperature is in the range of 70–85°F and in areas where the average rainfall is more than 40 inches per year. Lack of vegetation and a hard, clay-like soil adversely affect the development of the parasite. The filariform (thread-like) larvae lurk on vegetation or moist soil awaiting contact with a warm-blooded animal. The barefoot child is a likely candidate to provide the opportunity for the larva to contact bare skin, the portal of entry for the parasite. The eating habits of various population groups have a bearing on the incidence and persistence of certain parasitic diseases. The consumption of uncooked vegetables, particularly leafy vegetables, where human excreta have been used as fertilizer ("night soil") is a mode of transmission of the protozoal infections of amebiasis and giardiasis and of the helminthic infections of ascariasis and trichuriasis. The ingestion of raw fish may lead to anemia caused by infection with the fish tapeworm or to liver and gastrointestinal tract pathology caused by flukes. Raw beef may transmit the beef tapeworm and *Toxoplasma* organisms and, indi-

rectly, the pork tapeworm and the muscle worm. The distribution of African sleeping sickness is restricted to areas inhabited by the vector of the disease, the tsetse fly. The poor nutritional status in underdeveloped countries contributes to the severity of parasitic infections and to physical and mental retardation.

THE NATURE OF PARASITIC DISEASE

It is apparent from the incidence of infections that the parasitization of humans is common. The reactions of the host are often imperceptible or slight. The distinction is frequently made therefore in medical parasitology between parasitic infection and parasitic disease. In the average parasitized individual there are no signs or symptoms, or they are few or slight. That person has a parasitic infection. When there are overt signs and symptoms, when there is clear-cut clinical evidence of pathological changes, parasitic disease exists.

The occurrence of disease is often correlated with the number of parasites present and the complicating effects of multiparasitic infection, bacterial infections, malnutrition, age, and immune status. Many people in the tropics and subtropics, where parasites abound, suffer a multiplicity of parasitic infections. The triad of roundworm (ascariasis), whipworm (trichuriasis), and hookworm infection within the same person is not uncommon. Other parasitic combinations also occur. Singly, the infections might be comparatively benign.

Asymptomatic infections sometimes escalate into serious chronic disease. In chronic Chagas' disease, the patient may have heart disease or enlargement of the esophagus or colon years after the inception of an asymptomatic infection. Severe parasitic disease early in life may lead to retardation of growth and mental retardation, especially when compounded with malnutrition.

The pathogenesis of parasitic disease is varied. In addition to tissue invasion and destruction and toxic products, parasites create symptoms and pathology by obstruction, exertion of pressure, competition for host nutrients, and induction of hypersensitivities. The toxins are not devastating, as are the bacterial exotoxins.

Parasites that have definite tissue or organ affinity sometimes cause infections beyond the confines of the tissue or organ where they customarily localize. *E. histolytica*, for example, typically inhabits the gastrointestinal (GI) tract. It occasionally infects the brain and other vital organs, where it threatens life. To understand the symptoms and pathogenesis of some parasitic infections, it is necessary to know about the migrations within the body that are the normal mode of spread of some parasites. In trichinelliasis, for example, the early manifestations appear in the GI tract during the time adult females are producing live larvae. In heavier adult-stage infections, it appears that the patient has acute food poisoning. There may be fever, malaise, diarrhea, vomiting, and other GI complaints. In the next stage, larval migration, the chief complaint stems from myositis (inflammation of the muscles). The symptoms depend on the muscles involved. There may be difficulty in breathing, speaking, use of extremities, chewing, eye movement, and so forth. The larvae eventually become encysted in skeletal muscle, but death of the larvae in other tissues can result in pathology of those tissues. Persistent swelling of the extremities and of the area around the eyes (periorbital swelling) commonly occurs during the larval stage. There may be central nervous system (CNS) disturbances, congestive heart failure, and general wasting owing to loss of appetite. So many signs and symptoms accompany trichinelliasis that it is sometimes referred to as the *great imitator*. Other parasitic diseases have similar spectra of symptoms and pathology.

DIAGNOSIS OF PARASITIC INFECTION

Definitive diagnosis of parasitic infections depends largely on laboratory findings. Diagnosis based solely on clinical signs and symptoms is rarely possible or justified without laboratory confirmation, especially in nonendemic areas. The symptoms of parasitic disease are too diverse, too nonspecific, to lead to accurate clinical diagnosis. Parasitic in-

fection, especially exotic parasitic infection, may not be suspected in areas where parasitic diseases are not prevalent. Inquiry into the geographical and dietary history of the patient may reveal the opportunity for parasitization. Has the patient resided in or visited areas where parasitic infections are endemic within the incubation period? Does the patient recall an insect bite? Did the patient eat raw fish or uncooked vegetables?

Direct Examination

The major mode of laboratory identification is morphological identification, that is, recognition of the various forms of the parasite as they appear in clinical specimens. The diagnostic procedures of culturing, biochemical and serological tests, and animal inoculations that are commonly used in bacteriology and virology are much less frequently used in routine diagnosis of parasitic infections. The microscope is extensively used in the laboratory identification of parasitic infections because the forms most often sought are the microscopic cysts, trophozoites, and tissue forms of the protozoa and the typical, distinctive ova and larvae of the helminths.

Identification of Intestinal Parasites

The GI tract is the site of infection for many parasites, or it is the site where the infection is initiated with subsequent migration from the tract. Some of the organisms, particularly the trophozoites of certain protozoa, are frail. Speed and care in the handling of specimens are required so that the diagnostic morphological characteristics do not become altered or vanish before examination is completed. The laboratory should examine at least three fecal specimens serially collected at 24- to 48-hour intervals. Fecal specimens, collected in dry, clean containers, are first grossly inspected for blood, mucus, adult forms of worms, and consistency. Stools that are liquid or that contain blood or mucus should be kept warm and examined as soon as possible after evacuation. Most of the laboratory procedures are directed toward making suitable preparations for microscopical examination. These include unstained or supravitally stained (stained in the living state) wet mounts and fixed and stained smears. Concentration methods are sometimes employed before the slides are made. Concentration methods include comminution of the feces (breaking up through screens or gauze) followed by sedimentation, and flotation techniques in which the cysts of protozoa or the ova of the helminths descend to the bottom of the tube or float to the surface of the diluting fluid. Wet mounts or smears are prepared from the sediment or from the supernate and examined microscopically.

Blood Smears

Wet films or blood films are prepared to detect parasites such as trypanosomes, leishmanias, microfilarias, and plasmodia (malaria parasites). In the diagnosis of malaria, for example, films are prepared as thin smears and thick smears and are stained. The red blood cells (RBC's) remain intact in thin smears to better reveal the typical morphology of the infecting parasites and their effects on the RBC's. In thick smears the blood is not spread out into a monocellular film as in the thin smears. The RBC's are laked (dehemoglobinized by hypotonic lysis). The parasites in this concentrated state are less likely to be overlooked by the trained observer.

Examination of blood smears may disclose eosinophilia. Eosinophilia is a common finding in helminthic (worm) infections.

Tissue Examination

Wet mounts, stained tissue sections, and smears are also prepared from other body fluids and tissues. The material for such preparations is obtained by scrapings of skin and mucosal lesions, by biopsy, and by aspiration of the fluids of ulcers, abscesses, and bone marrow.

Unique Techniques

Several unique procedures for obtaining specimens merit special mention. A technique for sampling duodenal contents introduced in 1970 avoids the previously used, patient-discomforting method of duodenal intubation. A number of parasites establish themselves in the duodenum. In the new tech-

nique, called the Entero-Test method, the patient swallows a gelatin capsule that is packed with a nylon thread 140 cm long. The free end of the thread is fastened to the patient's face. After the capsule dissolves, the thread is carried by peristaltic action to the duodenum. It is gently retrieved after 4 hours. The droplets of duodenal contents on the distal end of the thread (composed of porous material) are then examined for parasites. Other unique techniques are those of xenodiagnosis, to be described under American Trypanosomiasis (Chagas' Disease), and the transparent tape contact method, to be described under Enterobiasis (Pinworm or Seatworm Infection).

Serology of Parasitic Infection

The use of serological tests in parasitic disease is increasing. The sensitivity and specificity of the tests are being constantly improved. Commercially prepared reagents are becoming available so that hospitals and clinics can now perform a number of their own serological tests rather than ship specimens to central government diagnostic laboratories as formerly. Not all parasitic diseases elicit an appreciable antibody response. Generally speaking, the immune response is greater with parasites that invade the tissues than with those that remain in the lumen of the intestine or those that are ectoparasitic (live on the outside of the host). Serological tests are especially useful in extraintestinal amebiasis, toxoplasmosis, Chagas' disease, visceral leishmaniasis, trichinelliasis, schistosomiasis, visceral larva migrans, and cysticercosis.

CONTROL AND TREATMENT OF PARASITIC INFECTION

The control of parasitic infection requires an understanding of how infections are acquired and the pathogenesis and clinical manifestations of the parasitic diseases; the role of hosts, especially intermediate hosts, including the environmental circumstances that determine the intermediate host's ecological niche; and the availability of control measures and the realistic chances of applying those measures. The ideal solution would be to eradicate parasitic infection, as was achieved with the virus disease smallpox, but currently that is not probable, especially in the absence of antiparasite vaccines. Eradication of many of the parasitic diseases would entail the expensive and formidable task of totally regulating intermediate hosts (insects, molluscs) and strictly managing environmental conditions (breeding places of insects, soil contamination with excreta). A reasonable goal with helminth infections, for example, is to reduce infectious doses by a combination of measures. Helminths do not multiply in the form of adults in the definitive human host, and so with a reduced infectious dose the worm burden would oftentimes not reach the level that causes disease. Ways to reduce the infectious dose include the application of insecticides, molluscicides (snail killers), and other agents that destroy intermediate hosts and vectors; managing environmental conditions; making foods and water supplies safe for consumption; and the use of therapeutic and prophylactic drugs in those populations or segments of populations that experience the highest rate of infection.

Public health agencies in the industrialized nations and in population centers in developing countries have largely been able to control parasitic disease through rigid standards for water supplies, sanitary disposal of wastes, inspection and sanitary packaging of foods, and control of arthropod vectors. Parasitic infections are common in the tropics, where there is the combination of suitable hosts, suitable climatic and geographical conditions, and a population group that suffers many deprivations. Control programs in the deprived areas must pay heed to sociocultural conditions and whenever possible include efforts to improve living conditions.

Despite the grim statistics of the incidence of parasitic infections, somewhat checkered progress is being made in reducing parasitic disease. Medical science has the knowledge and the medical means to improve the control of many of the infections. The World Health Organization (WHO) is deeply involved in attempting to control parasitic infections.

Travelers to places where parasitic infections are

endemic, especially when the itinerary or temporary residence is away from the urban areas, should be knowledgeable about the types of foods, beverages, insects, and other factors that are involved in the transmission of endemic infectious diseases. In addition to the required immunizations, recommended prophylactic drugs and immunizations should be given thoughtful consideration. The Centers for Disease Control (CDC), Atlanta, Georgia, annually publishes *Health Information for International Travel.*

Immunization

Development of vaccines for some parasitic diseases is underway (e.g., against malaria), but problems that stem from the characteristics of parasites make the attainment of an effective vaccine generally more difficult than with bacterial and virus vaccines: The parasites are antigenically very complex, they appear in different forms (trophozoites and cysts or adult worms, larvae, and eggs), they may continually change surface antigens (see *Trypanosomiasis*), and assessment of the immunological response of the host is difficult. As previously noted, lumen-dwelling parasites and ectoparasites generally do not evoke a pronounced, consistent, persistent immunological response.

Chemotherapy

Chemotherapy ordinarily should not be undertaken when the diagnosis of parasitic disease is provisionally based on clinical signs and symptoms or solely on serological findings. Furthermore, some of the antiparasitic drugs, especially those used for tissue infections, are toxic, and so the need for therapy must be weighed against the toxicity of the drug.

The effects of the antiparasitic drugs, as might be expected, are varied. Some are curative; they terminate the parasitosis. Some destroy the adult form of the parasite but are not able to eliminate the cysts or larvae. Some halt the multiplication of the parasite, an action which can be tantamount to curing the disease but not ridding the patient of the infection. Some are palliative, that is, they lessen the patient's discomfort.

A number of broad-spectrum drugs have been developed in recent years that are highly effective, particularly against lumen-dwelling parasites. They are well tolerated, easily administered, and of a comparatively low cost. The drugs include mebendazole, thiabendazole, levamisole hydrochloride, and pyrantel pamoate. They are especially useful as antihelminthic agents. Some of the recently developed drugs, although commercially available, are regarded as investigational for certain parasitic diseases. Others are strictly investigational and are available only from the CDC's Parasitic Disease Drug Service.

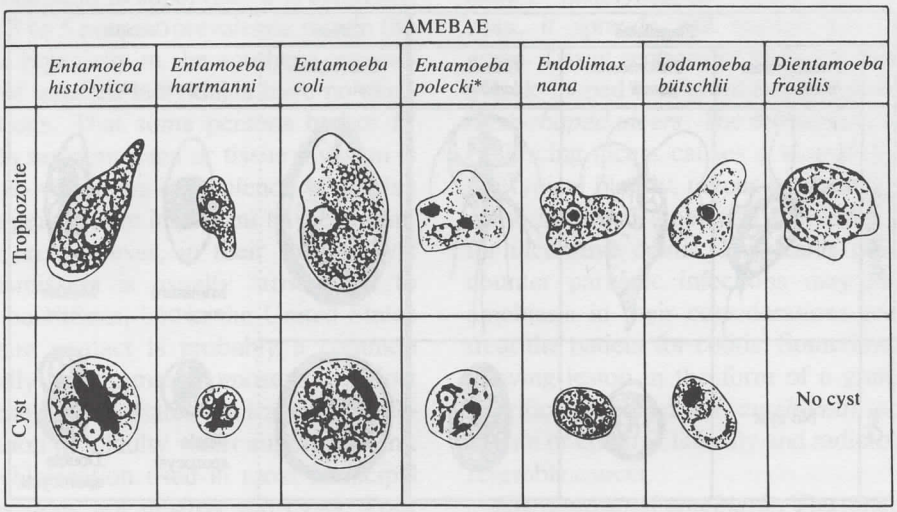
There is a need for drugs to treat those diseases that develop into chronic, disabling forms: filariasis, leishmaniasis, schistosomiasis, trypanosomiasis, and others. Once chronic inflammatory responses have produced scar tissue, little can be accomplished by drug treatment to undo the damage. Palliative drugs (analgesics, corticosteroids) or surgical intervention may be the only form of treatment applicable once unalterable tissue damage has occurred.

The appearance of drug-resistant strains sometimes impedes progress in the treatment and control of a parasitic disease. A notable example is that of the plasmodial protozoon that causes falciparum malaria. Strains that do not respond to first-choice antimalaria drugs are constantly evolving and relentlessly spreading globally. Alternative drugs, which are not only few but also usually less effective than the prototype antimalaria drug chloroquine, must be given instead.

THE PROTOZOA

The parasitic protozoa are customarily divided into four groups, variously termed *subphyla, classes*, or *subdivisions*: Sarcodina, Mastigophora, Ciliata or Ciliophora, and Sporozoa.

Fig. 36-2. Protozoa found in human stool specimens. (From M. M. Brooke and D. M. Melvin, *Morphology of Diagnostic Stages of Intestinal Parasites of Man*. Atlanta: Public Health Service Publication #1966, Center for Disease Control, 1969.)



*Rare, probably of animal origin.

SARCODINA—THE AMEBAE

Amebae that regularly parasitize humans (Fig. 36-2), *Endolimax nana, Iodamoeba bütschlii, Entamoeba coli, Entamoeba hartmanni* (considered by some to be a nonvirulent strain of *E. histolytica*), and *Dientamoeba fragilis*, are commensal, nonpathogenic amebae that colonize the intestinal tract. They have a direct life cycle (no invertebrate or intermediate host) and are cosmopolitan (worldwide) in their distribution. They are important because they must be differentiated from the one intestinal amebic pathogen, *E. histolytica*. *Entamoeba gingivalis* appears in the oral cavity in trophozoite form only. It is of uncertain pathogenicity but is generally regarded as a nonpathogen whose numbers increase the more unhygienic the condition of the oral cavity.

Differentiation of the amebae is based on the minute morphological differences in the trophozoites and cysts. Among the microscopical features that are sought in differentiation are the size and shape of the cyst; the number of nuclei (the mature cyst of *Entamoeba coli*, for example, characteristically contains eight nuclei; *E. histolytica*, four; and *I. bütschlii*, one); the chromatin distribution on the periphery of the nuclei; the shape, size, and position of the chromatin in the center of the nuclei; and the cytoplasmic inclusions such as glycogen masses and chromatoid bodies. The trophozoites are examined for size, shape, type of motility, chromatin distribution characteristics, and cytoplasmic inclusions.

There are two genera of free-living amebae, *Naegleria* and *Acanthamoeba*, that do not require an animal host and occasionally cause serious infections of humans, especially of the CNS.

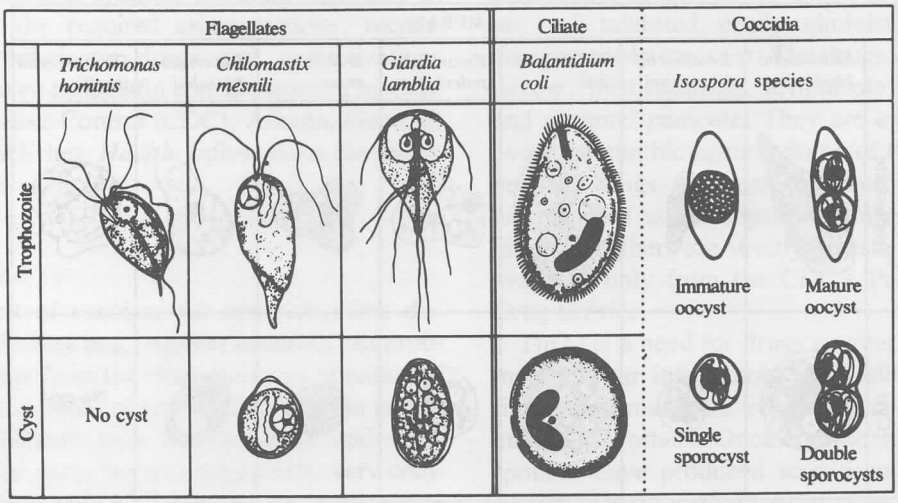


Fig. 36-3. Protozoa found in human stool specimens. (From M. M. Brooke and D. M. Melvin, *Morphology of Diagnostic Stages of Intestinal Parasites of Man*. Atlanta: Public Health Service Publication #1966, Center for Disease Control, 1969.)

MASTIGOPHORA— THE FLAGELLATED PROTOZOA

The Mastigophora at some time in their life cycle have whip-like flagella as locomotory organelles (Fig. 36-3). Some possess undulating membranes. Parasitic flagellates appear in the oral cavity (*Trichomonas tenax*), in the intestine (*Giardia lamblia, Trichomonas hominis, Chilomastix mesnili, Enteromonas hominis, Retortamonas intestinalis*), in the genitourinary tract (*Trichomonas vaginalis*), and in the circulation and tissues (the hemoflagellates and tissue flagellates of the genera *Trypanosoma* and *Leishmania*).

CILIATA OR CILIOPHORA— THE CILIATED PROTOZOA

The organelles for locomotion of the Ciliata are short, bristle-like cilia. The only ciliate that parasitizes man, *Balantidium coli*, is a large protozoon containing two types of nuclei, a macronucleus and a micronucleus.

SPOROZOA

Sporozoa have no special organelles of locomotion. They have a complex life cycle that includes sexual and asexual generation. The best-known sporozoite (animal spore form)-forming genera are *Plasmodium*, which contains species that cause malaria, and *Toxoplasma*.

PROTOZOAL DISEASES CAUSED BY THE AMEBAE

Amebiasis and Amebic Dysentery

The term *amebiasis* generally indicates a condition caused by *E. histolytica*, the only frank pathogen of the amebae. *E. histolytica* is best known as the causal agent of amebic dysentery. The clinical response to *E. histolytica* infection, however, is vari-

able. The ameba is worldwide in distribution. The greater incidences and the more serious infections are usually found in warmer climates and in communities and institutions where personal hygiene is neglected or is difficult to supervise. It is estimated that there is a 3 to 5 percent prevalence rate in the United States, especially in the southern regions. The majority of infected individuals have no clinical manifestations. That some persons harbor *E. histolytica* with no symptoms or tissue invasion is probably due to variations in virulence of amebic strains. These persons are important to the continued transmission, however, in their role as cyst passers. Transmission is usually attributable to poor sanitary conditions, but in the United States person-to-person contact is probably a common mode, especially among male homosexuals. Most epidemics in the United States are traceable to fecal contamination of a faulty water supply system. The level of chlorination used in most municipal water supplies does not destroy the cysts. Poor sanitation, including the use of night soil (human excreta) as fertilizer, is mainly responsible for the high incidence of amebiasis in the tropics.

SYMPTOMS AND PATHOGENESIS. Overt infections are in the forms of a diarrhea that tends to be protracted, dysentery of varying severity, amebic abscesses that form in various organs, and an amebic granuloma (ameboma).

Intestinal amebiasis. The ameba appears to exist largely as a commensal in the colon. There is some question as to whether the parasite initiates disease on its own or invades and causes disease once tissue is damaged by other means. *E. histolytica* has gelatinase, hyaluronidase, amylase, and trypsin activities, but how these enzymes relate to the invasive behavior has not been clearly demonstrated. The species name implies tissue-dissolving activity. Some investigators postulate that the pathogenesis involves sensitization of submucosal tissue by previous invasions of trophozoites. Subsequent invasions initiate tissue-destructive inflammatory reactions and at the same time create conditions for colonization by newly arrived trophozoites. Recently, heat-labile material obtained from pure cultures of *E. histolytica* was shown to have cytotoxic and enterotoxic activity. This material could account for the diarrhea and tissue injury that occur in amebiasis. Whatever the pathogenic mechanism, once *E. histolytica* has breached the intestinal mucosa, it spreads and undermines the subjoined areas. Tissue sections of such affected areas reveal a flask-shaped configuration, giving rise to the term *flask-shaped ulcers*. The formation of multiple and coalescing ulcers causes a violent dysentery with the typical bloody, mucus-containing stools. Clinically, intestinal amebiasis can easily be mistaken for ulcerative colitis. Physicians who seldom encounter parasitic infections may fail to include amebiasis in their considerations and mistakenly treat the patient for colitis. Sometimes a space-occupying lesion in the form of a granuloma (more specifically called an *ameboma*) occurs in the cecum or colon. Clinically and radiologically it may resemble cancer.

Extraintestinal amebiasis. The amebae may penetrate all the way through the intestinal wall and be carried via mesenteric venules and lymphatic vessels to other organs to cause extraintestinal amebiasis. The organ most likely to be so affected, usually by amebic abscesses, is the liver. Amebic liver abscess is a serious public health problem in developing nations. Other sites of amebic invasion are the lungs, skin, brain, and pericardium.

LABORATORY DIAGNOSIS. Bloody mucous stools that are examined within a half hour after evacuation may contain the living, motile trophozoites. Trophozoites may also be found in ulcer scrapings, rectal biopsy specimens, and aspirated material. Cysts may be found in formed stools. Several different fixatives and stains are applied to smears and sections of these materials in preparation for microscopical examination. It is necessary to be able to differentiate the trophozoites or cysts of *E. histolytica* from the other intestinal amebic parasites (see Fig. 36-2). The structure of the cyst nuclei and the presence of red blood cells within the trophozoites (hematophagous trophozoites) are among the more distinctive characteristics of *E. histolytica*. The diagnosis of extraintestinal amebiasis rests on the

clinical evaluation along with laboratory findings. Cultivation of aspirated material and especially serological tests are used in the diagnosis of extraintestinal amebiasis. Serum antibody can be detected by commonly used serological techniques. The serological tests of choice are the indirect hemagglutination test and a gel diffusion test. The tests are positive in 93 to 97 percent of the cases of extraintestinal amebiases. Specificity is high with the use of pure antigen.

TREATMENT AND CONTROL. A variety of chemotherapeutic agents are used to treat amebiasis: emetine, iodine-containing compounds such as iodoquinol (diiodohydroxyquin) and chiniofon, tetracyclines, and chloroquine. The choice of drug is determined by the clinical type of the infection. Metronidazole or iodoquinol are applicable to the various types of infections: asymptomatic, mild to severe intestinal disease, and hepatic abscesses. Diloxanide furoate (furamide) is used for asymptomatic cyst passers.

Control of *E. histolytica* infections depends on improved hygiene, sanitation, and water treatment; isolation of patients; and education about the risks of anal-oral contact.

Dientamoeba fragilis

D. fragilis (see Fig. 36-2) has been categorized as a commensal intestinal ameba and is still so regarded in most quarters. Some protozoologists, however, now consider it a flagellate (Mastigophora) because its nuclei resemble those of trichomonads. *D. fragilis* is being incriminated as a pathogen of children. It causes acute diarrhea that is accompanied by abdominal pain, loss of appetite, cramps, and mild dehydration. There is no known cyst stage for this delicate (*fragilis*) protozoon. Metronidazole is effective against it.

Primary Amebic Meningoencephalitis

The causative agent of primary amebic meningoencephalitis (PAM), a usually rapidly fatal disease that was first described in Australia in 1965, is a free-living, soil-water ameboflagellate (an ameba that can transform into a flagellate), *Naegleria fowleri*. The infection is typically seen in young, healthy persons and is usually associated with a history of swimming in warm bodies of fresh water that are either natural or man-made. The parasite probably gains entrance to the brain via the nasal passages and from there along olfactory nerve fibers through the cribriform plate to the olfactory bulbs. Spread within the gray matter and multiplication of the parasite are rapid, and unless early diagnosis is made and treatment initiated, the patient dies within about a week to 10 days from the time of exposure. Inflammation and tissue damage can lead to severe frontal headache; disturbances of smell, taste, and vision; anorexia; nausea; fever; and stiff neck. The patient may experience or exhibit convulsions, irrational behaviour, seizures, and coma. All in all, the patient's condition resembles fulminant septic meningitis. The CDC in Atlanta has recorded 123 cases worldwide with only three survivors as of July 1978. Table 36-1 lists the seven confirmed cases and one suspected case of PAM that occurred in the United States and Puerto Rico in 1980. Diagnosis is made by identifying the living or stained amebae in cerebrospinal fluid or in postmortem brain tissue. No satisfactory treatment for PAM exists. One patient, an 8-year old girl from California who swam in a hot springs pool that had a water temperature of 105°F was diagnosed early and was treated successfully with amphotericin B, miconazole, and rifampin.

Granulomatous Amebic Meningoencephalitis and Disseminated Acanthamebiasis

Free-living amebae of the genus *Acanthamoeba* probably enter the human host via the skin or lung and from there may spread to the CNS by the hematogenous route. Infection occurs primarily in the immunocompromised and the chronically ill. Corticosteroid therapy, treatment with cytotoxic drugs, and possibly alcoholism predispose to infection. CNS involvement follows a subacute course of days to months and is in the form of foci of necrotizing encephalitis, which microscopically have elements of a chronic inflammatory response known as granuloma formation—hence the name *granulomatous amebic encephalitis* (GAE). Clini-

Table 36-1. Cases of *Naegleria*-type Primary Amebic Meningoencephalitis Reported to CDC, 1980

Reporting state or territory	Age	Sex	Place of exposure	Date of exposure	Approximate date of onset	Date of death	Method of confirmation
California	14	F	Colorado—stockpond, Oklahoma—lakes	6/22–7/2	7/6	7/15	CSF exam
Florida	6	M	East Florida—lake	?	7/22	8/1	CSF exam
Florida	15	M	Central Florida—lake	7/21–7/25	7/29	7/31	None
Florida	12	M	West-central Florida—lakes, ditch	7/28–8/1	8/9	8/20	CSF exam
New York	10	M	East-central Florida—lake	7/31–8/6	8/11	8/23	CSF exam and culture
Puerto Rico	17	M	Northeast Puerto Rico—lagoon	?	6/23	6/26	Brain tissue (IFA,* postmortem)
Texas	37	F	Southeast Texas—lake	7/6–7/12	7/11	7/14	Brain tissue (postmortem)
Texas	23	F	South Texas—lake	7/5	7/11	7/16	Brain tissue (postmortem)

*Indirect-fluorescent-antibody test.
Source: From *MMWR* 29:405, 1980.

cal manifestations of CNS involvement are largely determined by the site of the lesion or lesions but often include signs and symptoms of global CNS dysfunction such as headache, mental status changes, and seizures. Skin lesions that either represent the primary infectious site or result from dissemination via the hematogenous route are the other principal clinical manifestations.

PROTOZOAL DISEASES CAUSED BY THE FLAGELLATES

Giardiasis (Lambliasis)

Giardia lamblia is a flagellated intestinal protozoon that was regarded as a borderline pathogen for many years. It is now known to cause significant morbidity. It is the causal agent of the most common waterborne diarrheal disease in the United States. The trophozoite, when viewed from the ventral side, is a pear-shaped organism 9 to 16 μm in length, 9 to 12 μm in width, and 2 to 5 μm in thickness. It contains four pairs of flagella; two prominent nuclei; a stiff, rod-like structure, the axostyle; and an anterior, oval, concave, adhesive (sucking) disk that occupies most of the flat ventral surface (Fig. 36-4). It has a falling-leaf type of movement. The tough-walled cyst, which is the infective stage, is an ovoid form 9 μm by 12 μm containing two to four nuclei and many of the structures that are seen in the trophozoite.

SYMPTOMS AND PATHOGENESIS. The spectrum of infection ranges from asymptomatic colonization to a severe persistent malabsorption syndrome (Table 36-2). The parasite is found in the upper part of the duodenum and jejunum. It does not ordinarily invade the tissues but is a lumen dweller. Watery diarrhea, flatulence, and abdominal cramps are

Fig. 36-4. Scanning electron micrograph of *Giardia muris* trophozoites in a mouse intestine. A. Dorsal view. B. Ventral view. C. Adhesive disk on the ventral surface. D. Circular mark left by the adhesive disk on the columnar cell microvilli of intestine (×8026). (From R. L. Owen, P. C. Nemanic, and D. P. Stevens, Ultrastructural observations of giardiasis in a murine model. *Gastroenterology* 76:757, 1979.)

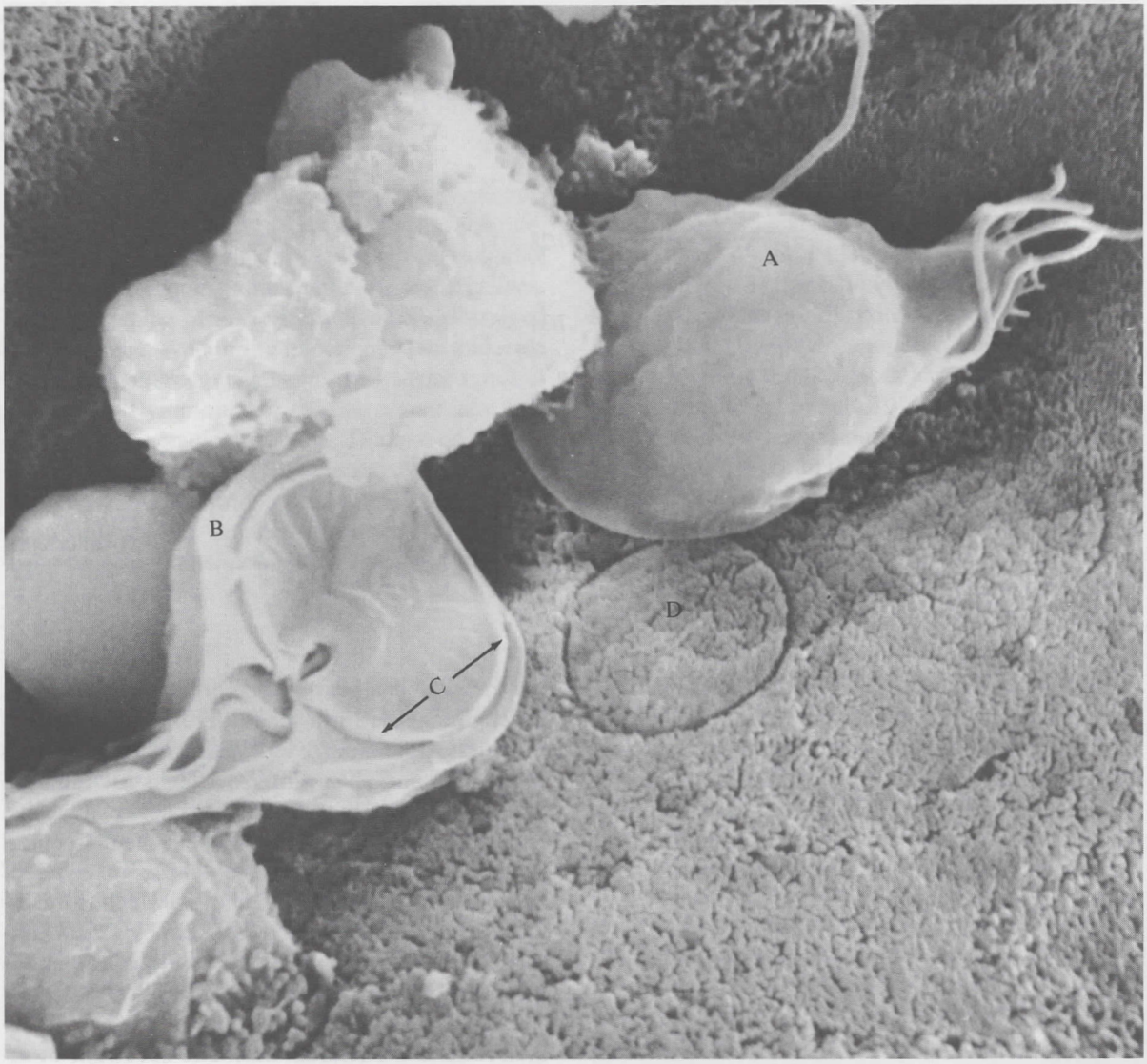


Table 36-2. Symptoms Associated with Different Stages of Giardiasis

Acute	Subacute	Chronic
Diarrhea of sudden onset, with explosive, watery, often foul-smelling stools	Intermittent attacks of mushy, foul-smelling stools with some evidence of steatorrhea	Periodic, brief episodes of loose, semisolid, foul-smelling stools, with constipation occasionally occurring between attacks
Marked flatulence and abdominal distention	Greater-than-normal flatulence, abdominal distention	Passage of foul-smelling flatus
Abdominal cramps, often midepigastric	Midepigastric or more generalized abdominal pain or substernal burning	Abdominal distention
Nausea, anorexia, occasional vomiting	Belching of foul-smelling gas	Some weight loss
Chills, low-grade fever, headache	Anorexia, weight loss	Intermittent attacks of lassitude and malaise
Belching	Fatigue, lassitude, malaise	
Generalized weakness	Urticaria (few cases)	

Source: N. Amin, Giardiasis: A common cause of diarrheal disease. *Postgrad. Med.* 66:151, 1979.

common complaints in acute infections. In subacute and chronic infections there may be episodic diarrhea; malodorous, bulky, greasy stools; abdominal distention; flatus (gassiness); loss of appetite; and weight loss. The malabsorption syndrome, in which there is interference with the absorption of fats, carbohydrates, and vitamins, includes wasting, reduction in serum albumin (hypoalbuminemia), diarrhea, and steatorrhea (high fat content of feces). Possible mechanisms through which *Giardia* damages intestinal epithelial cells include formation of a tightly joined network of parasites on the mucosal surface that interferes with the passage of nutrients, inflammation that leads to cell injury, damage to microvilli by the overlying trophozoites (electron microscopical studies show deformation of microvilli), alteration of bacterial flora that are coinfectors, production of as-yet unidentified toxins, and competition for nutrients.

EPIDEMIOLOGY. Infection occurs via the ingestion of cysts from fecally contaminated sources. Two known routes of transmission to humans are contaminated water supplies and person-to-person spread. Waterborne outbreaks have occurred via community water supplies in over two dozen municipalities that use surface water sources. Failures in the filtration aspects of the water purification systems appear to be the determining factor in the failure to eliminate the parasite. Chlorine levels routinely used to disinfect municipal water supplies (0.4 mg/L free chlorine) do not kill *Giardia* cysts in the usual exposure times. In 1979 waterborne outbreaks occurred in Estes Park, Colorado; Plumas County, California; Zig Zag and Government Camp, Oregon; and Bradford, Pennsylvania. There were 407 confirmed cases of giardiasis among the residents of Bradford. Animal reservoirs are suspected to exist because some infections have been traced to the drinking of water (e.g., from mountain streams) in areas unoccupied by humans. *Giardia*-infected beavers are leading candidates as an animal reservoir since they have been observed frequently in waters upstream from filtration plants that were implicated in community water supply outbreaks. Transmission through person-to-person contact is suggested by the observations of high frequency of giardiasis among children in day-care centers, by the 15 to 30 percent incidence in children below the age of 2 in developing countries, and by the incidence in promiscuous male homosexuals. It is estimated that 2 to 20 percent of the population in the United States harbors this protozoan, depending on the age group and community surveyed.

LABORATORY DIAGNOSIS. Diagnosis is made by finding trophozoites or cysts in feces, intestinal aspirates, or material retrieved via the Entero-Test method (see Unique Techniques earlier in this chapter). The trophozoite is found in feces only in cases of profuse diarrhea because this more delicate form is usually destroyed in the sojourn from the upper intestine. The physician should consider the possibility of giardiasis in patients who have diarrhea that persists longer than 1 week, especially if the diarrhea is accompanied by any two of the following: foul-smelling stools, bloating, abdominal cramps, loss of appetite, or loss of weight. A history of the travels of the patient, a history of drinking untreated water from surface bodies of water, and community outbreaks of diarrhea should alert the physician to rule out giardiasis.

TREATMENT. Quinacrine hydrochloride is the drug of choice. Alternative reliable drugs are metronidazole and furazolidone for children. Adequate treatment with these drugs usually results in prompt resolution of symptoms.

Trichomoniasis

Trichomonads are globular protozoa possessing three to five anterior flagella (see Fig. 36-3). There is a single prominent oval nucleus. An internal, stiff, rod-like structure called an *axostyle* runs the length of the protozoon and emerges posteriorly as a caudal spine. An undulating membrane, a thin filmy structure, extends along the longitudinal axis. Trichomonads appear only in the trophozoite form, the transmissible form. Three morphologically similar *Trichomonas* species are found in humans: *T. tenax, T. hominis*, and *T. vaginalis*. Differentiation among the three is possible on morphological and cultural grounds. Each has a specific habitat in humans.

TRICHOMONAS TENAX AND *TRICHOMONAS HOMINIS*. *T. tenax* and *Entamoeba gingivalis* are the only animal parasites that have the oral cavity as their regular habitat. They apparently occur in only a low percentage of healthy mouths. Most investigators regard them as scavengers or commensals that flourish best when there is disease of the periodontium, the supporting soft tissue of the teeth. Neither forms demonstrable cysts, and neither appears to have tissue invasive tendencies. Transmission probably occurs through saliva-soiled fomites (e.g., tableware) and through kissing.

T. hominis, found in the human intestinal tract, is clinically unimportant.

TRICHOMONAS VAGINALIS. *T. vaginalis* is regarded as a pathogen and causes an estimated 2 to 3 million cases of sexually transmitted disease annually. Hygienic practices and promiscuity have a bearing on the incidence of infection.

Infections in Women. *T. vaginalis* is one of the three main causes of vaginitis, the other two being the bacterium *Gardnerella vaginalis*, which causes a frothy discharge and a fishy odor, and the endogenous yeast, *Candida albicans*, which causes vulvovaginal irritation with itching and a curd-like discharge. *T. vaginalis* infections are normally limited to the vagina, cervix, and vulva. Only about 15 percent of those infected experience symptoms. Heavy infections are likely to be accompanied by pruritus (itching) and intense inflammation that produces a thick, foul-smelling discharge that is sometimes bloody and occasionally frothy. Cervical involvement may cause dyspareunia (painful coitus). There may be pruritus and chafing of the vulva. Transmission is generally by sexual intercourse, although it can occur via fomites such as unclean toilet facilities and contaminated garments.

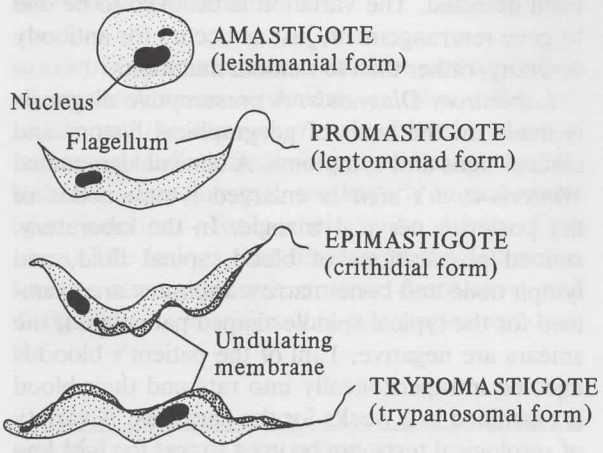
Infections in Men. Men, usually symptomless, may have urethritis, epididymitis, or prostatovesiculitis. They may note a slight discharge, especially with the first urination in the morining. There may be dysuria (painful urination) and increased urination.

Diagnosis. Saline wet mounts are prepared from genitourinary tract specimens and examined microscopically for the typical jerky, swift movements of the living trichomonad. The edges of the undulating membrane look like a cogwheel as they come into and move out of focus. Stained trichomonads are not uncommonly discovered in routine

Papanicolaou (Pap) smears prepared for cancer detection.

Treatment. The approval of metronidazole in 1963 revolutionized treatment. Metronidazole is taken orally, and sometimes a metronidazole-containing vaginal suppository is prescribed simultaneously. Before metronidazole, efforts were made to treat intractable infection by a variety of local applications that were intended to reduce the number of trichomonads and to return the vaginal pH to its normal level of 3.8 to 4.4. (*T. vaginalis* infections raise the pH of the vagina.) Methods included acid douches, reintroduction of acid-forming *Döderlein's bacillus* (a lactobacillus), drug insufflation (introduction of powdered or vaporized drugs), and drug-impregnated inserts. Regular sex partners of women with vaginal trichomoniasis should also be treated with metronidazole.

Fig. 36-5. The body forms of members of the genera *Trypanosoma* and *Leishmania*.



Trypanosomiasis

There are seven species of blood and tissue flagellate protozoa (collectively referred to as *hemoflagellates*) that infect humans. They appear in two genera: *Trypanosoma* and *Leishmania*. The parasites live alternately in vertebrate hosts and in bloodsucking insects where they undergo a cyclic development. There are four distinct cyclic morphological types: the amastigote (leishmanial form), the promastigote (leptomonad form), the epimastigote (crithidial form) and the trypomastigote (trypanosomal form) (Fig. 36-5).

AFRICAN TRYPANOSOMIASIS (AFRICAN SLEEPING SICKNESS). The *Trypanosoma* species that is medically and economically important is *T. brucei*. It contains three subspecies, *T. brucei gambiense* (chronic sleeping sickness in humans), *T. brucei rhodesiense* (acute sleeping sickness in humans), and *T. brucei brucei* (nagana in cattle). Tsetse flies transmit *rhodesiense* and *gambiense* to humans. A wide range of wild game animals and domestic animals serve as reservoir hosts for *T. b. rhodesiense*. *T. b. gambiense* supposedly is not maintained in nature by animal reservoir hosts, so its cycle involves only humans and tsetse flies.

The parasites first lodge in the local tissues where they were introduced by the bite of the tsetse fly. After 2–3 days there is local inflammation and itching, followed about 4 days later by the development of a chancre. Approximately a week or two after infection the parasite is detectable in the blood. Episodes of parasitemia that last for 2–3 days follow at intervals of 2–10 days. Headaches, joint pain, malaise, and enlarged lymph nodes and spleen accompany the recurring episodes of parasitemia. The second stage of the disease, the sleeping sickness phase, involves the CNS. Insomnia, irritability, personality changes, and decreasing mental acuity precede a gradual loss of CNS function. In the advanced stages the victim suffers convulsions and becomes somnolent and comatose before eventually expiring from malnutrition and secondary infection. Untreated *rhodesiense* infections run a course from weeks to months; *gambiense* from months to years.

Continual Variation of Surface Antigens as a Means of Evading Existing Antibody. The relapsing parasitemia and the longevity of infections despite an elevated IgM response by the patient were for many years unexplained. Observations on *T. b. brucei* infections in experimental animals provided the answer. *Trypanosoma* parasites evade existing

antibodies by a continual variation and shedding of surface coat antigens. Many antigenic types sequentially derived from the same organism have been detected. The variation is believed to be due to gene rearrangement, just as occurs for antibody diversity, rather than to random mutation.

Laboratory Diagnosis. A presumptive diagnosis is made on the basis of geographical history and clinical signs and symptoms. A clinical sign named *Winterbottom's sign* is enlarged lymph nodes of the posterior cervical triangle. In the laboratory, stained preparations of blood, spinal fluid, and lymph node and bone marrow aspirates are examined for the typical spindle-shaped parasites. If the smears are negative, 1 ml of the patient's blood is injected intraperitoneally into rats and their blood is examined in 2 weeks for the parasites. A variety of serological tests can be used to test for IgM levels. Indirect fluorescent antibody testing is the most commonly used technique.

Treatment and Control. Suramin sodium administered intravenously is used for the bloodstream and lymphatic stage and melarsoprol for late disease with CNS involvement. Control consists of medical surveillance, chemoprophylaxis, and vector control through the use of insecticides and modification of the tsetse fly principal habitat, namely, the dense vegetation along rivers and in forests.

AMERICAN TRYPANOSOMIASIS (CHAGAS' DISEASE). *Trypanosoma cruzi* is transmitted by various species of reduviid bugs that are known as *assassin bugs* or *kissing bugs* in the United States, as *barbieros* in Brazil, and as *vinchucas* in Spanish-speaking South America. They are called kissing bugs presumably because of their predilection for alighting on the face and lips. Human beings, domesticated animals, and wild animals are the most important vertebrate hosts. Cats and dogs serve as the main reservoirs for humans. The insect vectors and infected raccoons and opossums have been found in the southwestern and southeastern United States. It is estimated that 7 million people in South America and Central America are infected. The lower economic groups who dwell in thatched adobe huts are the population group most likely to be affected. The adobe huts provide breeding places for the insect vectors.

The parasites multiply in the tissues rather than in the blood. In the tissues (reticuloendothelial system [RES], heart, and CNS) they multiply in the leishmanial form, whereas in the blood they appear in the trypanosomal form. Infections are usually subclinical. In a small percentage of cases, usually in children, infection takes an acute form. About 50 percent of individuals develop a facial lesion at the site of entry of the parasite, often accompanied by unilateral palpebral edema (swelling of one eyelid). The palpebral edema of Chagas' disease is called *Romaña's sign.* About 10 percent of acute infections are fatal, usually because of infections of the heart or CNS. It is not unusual for the infection to remain asymptomatic for years and then to become gradually manifest as chronic Chagas' disease. The parasites localize in various tissues and organs. Infections of the heart, a typical site of localization, are a leading cause of cardiac disease and sudden death in endemic areas. Grossly enlarged hearts and congestive heart failure are characteristics of Chagas' disease. Another manifestation of chronic disease is dilation and enlargement of the esophagus and colon (megaesophagus and megacolon, respectively).

A positive geographical history, residence under conditions in which transmission is known to occur, and typical clinical signs and symptoms suggest American trypanosomiasis. The trypanosomal morphological form may be observed in stained blood films obtained during febrile stages of infection. The leishmanial morphological form may be found within macrophages that are aspirated from RES tissue. Cultivation on diphasic blood agar* or infection of mice and serological tests are also employed.

A definitive diagnosis is not easily accomplished. The parasite is scarce in circulating blood, the most accessible specimen. However, a sensitive, indirect

*Diphasic blood agar is a 5-ml beef infusion agar slant that contains 10 percent defibrinated rabbit blood; just before inoculation 2 ml of sterile Locke's (saline) solution is added to the tube.

technique, called *xenodiagnosis*, is used in areas where trypanosomiasis is endemic. Laboratory-reared reduviid bugs that are free of *T. cruzi* parasites ("clean bugs") are allowed to feed on the patient. Ten days later the rectal contents of the bugs are examined for active stages of the parasite.

Leishmaniasis

In definitive hosts, such as humans, *Leishmania* species appear as oval parasites 3 to 5 μm in length. They are intracellular parasites of macrophages. In culture they are motile flagellates. Transmission is predominantly by sandflies (*Phlebotomus* and *Lutzomyia* flies), the intermediate host. Reservoir hosts include dogs, cats, wild rodents, horses, sheep, and cattle. Four morphologically indistinguishable species—*L. donovani, L. tropica, L. mexicana*, and *L. braziliensis*—infect humans, producing visceral leishmaniasis, cutaneous leishmaniasis, and mucocutaneous leishmaniasis. There is considerable overlap or intergradation of clinical manifestations caused by the four species. Diagnosis of the leishmaniases is made by demonstrating the organisms in stained smears from lesions or in biopsy specimens. Aspirated specimens are cultured on diphasic blood agar or injected intraperitoneally into hamsters. Antimony sodium gluconate is the drug of choice for all four types of leishmanial infection.

VISCERAL LEISHMANIASIS (KALA-AZAR). Visceral leishmaniasis, or kala-azar, is found in foci in most tropical and subtropical countries. It is a diffuse parasitization of the entire RES, usually caused by *L. donovani*. Phagocytic cells are stuffed with the amastigote parasites, bringing about enlargement of the spleen, liver, lymph nodes, and other parasitized tissues and organs. There is an accompanying reduction of neutrophils (granulocytopenia), rendering the patient subject to intercurrent infections of the lungs and digestive tract that may be fatal.

CUTANEOUS LEISHMANIASIS. In cutaneous leishmaniasis (also called *Oriental sore*), an ulcerating lesion develops at the site of each inoculating insect bite. The ulcer may become secondarily infected. A permanent depigmented scar develops 9–12 months later in untreated cases. *L. tropica* is associated with the Old World infection; *L. mexicana*, with the New World version.

MUCOCUTANEOUS LEISHMANIASIS. The primary lesion of mucocutaneous leishmaniasis (also called *espundia* and *chichlero ulcer*) resembles the lesion of cutaneous leishmaniasis. The parasite may metastasize to secondary foci, particularly to mucocutaneous junctions—for example, in the vicinity of the nares. The lesions that develop at the secondary foci are often extensive, erosive, destructive, and disfiguring. *L. braziliensis* is the species responsible.

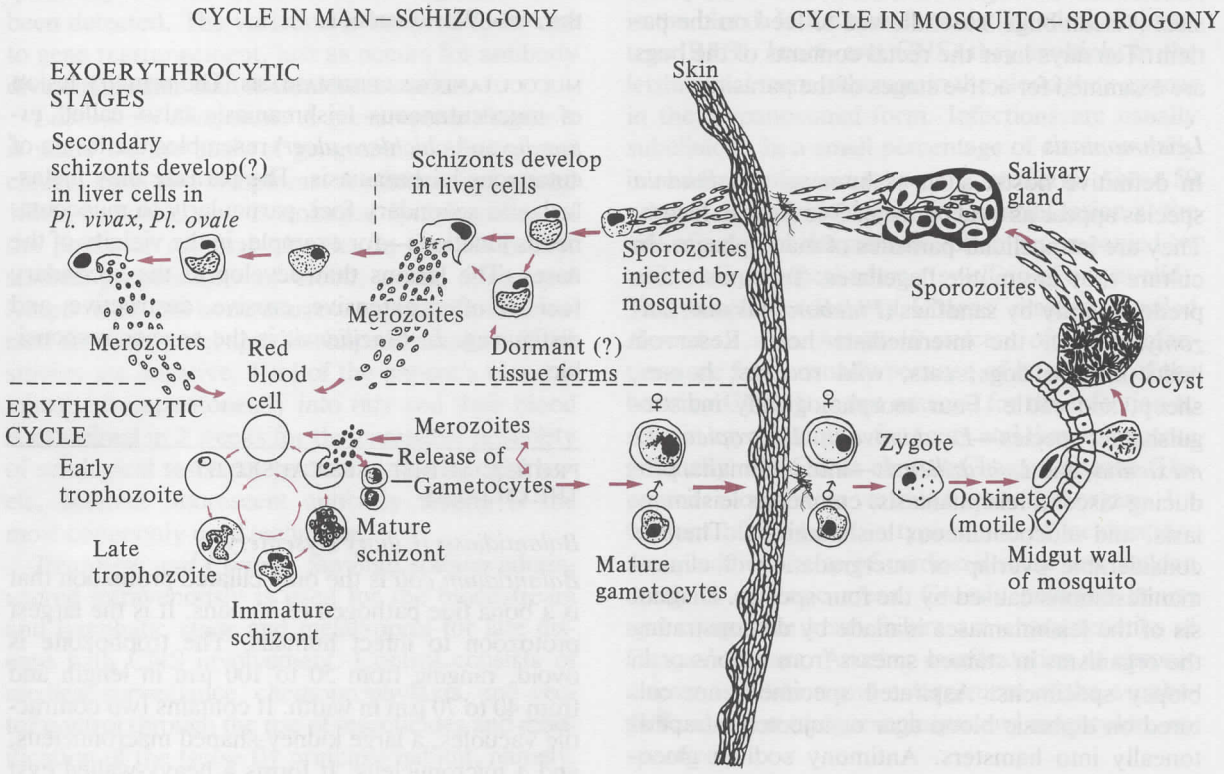
PROTOZOAL DISEASES CAUSED BY THE CILIATES

Balantidiasis (Ciliary Dysentery)

Balantidium coli is the only ciliated protozoon that is a bona fide pathogen of humans. It is the largest protozoon to infect humans. The trophozoite is ovoid, ranging from 50 to 100 μm in length and from 40 to 70 μm in width. It contains two contractile vacuoles, a large kidney-shaped macronucleus, and a micronucleus. It forms a heavy-walled cyst with markings on the surface where the shed cilia were located.

B. coli is a common parasite of swine and of several lower primates. From 60 to 90 percent of hogs harbor *B. coli* or *B. suis*. The latter, which does not infect humans, is the more common in hogs. Infection of people is rare, the chief symptom being diarrhea of varying severity. Occasionally a dysentery, termed *balantidial dysentery* or *ciliary dysentery*, develops. Diagnosis is based on the identification of the characteristic trophozoites and cysts in diarrheic or formed stools. Iodoquinol and oxytetracyclines are used for treatment.

Fig. 36-6. Malaria life cycle.



PROTOZOAL DISEASES CAUSED BY THE SPOROZOA

Malaria

At least five species of *Plasmodium* infect humans: *P. vivax, P. malariae, P. falciparum, P. ovale*, and *P. knowlesi*. Essentially, most of the information and incidence relate to the first three species. Falciparum malaria is regarded as the most serious form of malaria.

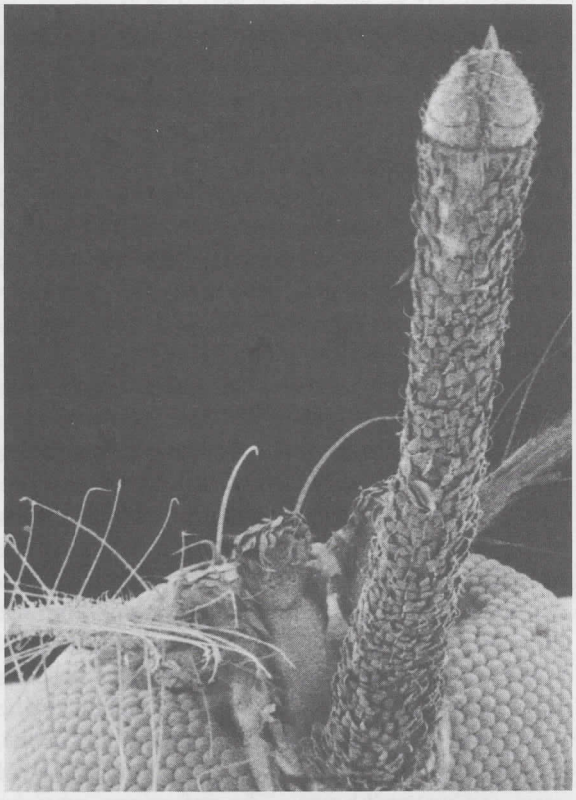
One of the truly major infectious diseases of humankind, malaria has among its many names *plasmodiasis, tropical fever, marsh fever, paludism, coastal fever*, and *ague*. It is largely limited to the tropics and the subtropics. Fifty percent of the world's population resides in malarious regions. It is estimated that 200 to 400 million persons contract malaria each year. According to the WHO, malaria is responsible annually for the death of 1 million children below the age of 5 in Africa. The incidence of malaria in the United States and Puerto Rico among civilians shows an upward trend from 139 cases in 1969 to 585 cases in 1978. Most civilian cases are imported through travelers and immigrants. Transmission from the imported cases has led to isolated episodes of malaria in nonepidemic areas (referred to as *introduced malaria*). Susceptible mosquitos and infected individuals whose blood contains gametocytes (sexual forms that mature into gametes) are required for transmission to occur in this situation. There is an exceedingly low probability of introduced malaria being further transmitted because the chances of mosquitoes becoming infected and surviving long enough for the

parasites to mature are minimal. Malaria transmitted by drug addict practices falls into the category of *transfusion malaria*. Such an infection, incidentally, is only in the erythrocytic cycle (see next section); that is, there is no liver stage to the infection.

LIFE CYCLE. A knowledge of the malaria life cycle (Fig. 36-6) aids in understanding the clinical events, the treatment, and the control measures. The two phases in the life cycle of the malarial parasite are sporogony and schizogony. The sexual phase, *sporogony*, occurs in the female *Anopheles* mosquito. It is initiated when the mosquito, while taking a "blood meal" from an infected individual, takes in red blood cells that contain the male and female gametocytes of the malaria parasite (Fig. 36-6, lower right). The gametocytes mature in the midgut of the mosquito and form zygotes. Growth results in the formation of oocysts, which come to locate on the outside of the mosquito stomach. Hundreds of sporozoites develop in each oocyst, whence they are released into the body cavity. Eventually they reach the salivary glands.

The asexual phase, known as *schizogony*, takes place in the vertebrate host. It is initiated when the sporozoite-laden saliva of the mosquito is introduced at the time of the mosquito bite (Fig. 36-7). There are two stages in the development of malaria once the sporozoites enter the body. Plasmodial infection is primarily an infection of the red blood cells. Before the infection of the red blood cells there is a liver stage; it is termed the *preerythrocyte stage*, and it may include exoerythrocytic cycles (see Fig. 36-6, upper left). The parasites are called *cryptozoites* (the hidden forms) in the liver stage. The entrance of the sporozoites into the liver cells begins the incubation period, which lasts 10–28 days, depending on the plasmodial species. *Schizonts* are formed. The liver cells rupture, yielding cytoplasm-enveloped schizonts (now called merozoites) that either infect other liver cells (to produce the exoerythrocytic cycle) or infect red blood cells. In falciparum malaria there is only one preerythrocytic liver cell infection. In malariae and vivax malaria the liver cell infection can be repeated

Fig. 36-7. Head of mosquito. The triangular tip that protrudes from the very end of the mosquito's proboscis is the part that actually penetrates a mammal's skin to reach the blood. On either side of the proboscis are the hemispheric eyes, each composed of several hundred individual facets (× 183, before 28% reduction). (From P. B. Armstrong, D. W. Deamer, and J. J. Mais, What's Biting You? *J. Natl. Hist.* June/July 1972.)



in the exoerythrocytic cycle. Knowledge of the exoerythrocytic cycle is important in chemotherapy for malaria.

The release of merozoites from the liver cells and their entry into red blood cells initiates the erythrocytic stage (see Fig. 36-6, lower left). The merozoites develop into trophozoites, which eventually segment to give rise to a new crop of merozoites. The merozoites infect other red blood cells. In falciparum malaria as many as 40 percent of the red blood cells become infected. The release of the

merozoites usually becomes synchronized; that is, infected red blood cells rupture at approximately the same time. The paroxysms of chills and fever experienced by the patient are closely related to that release. During the erythrocyte sequences some of the trophozoites develop into gametocytes. The ingestion of the gametocytes by the mosquito initiates the sporogenous phase.

SYMPTOMS AND PATHOGENESIS. The infected individual experiences the paroxysms of malaria in consonance with the synchronous release of the merozoites. In vivax malaria synchronous showers of merozoites are released every 48 hours. Vivax malaria is therefore called *tertian malaria* because the interval spans 3 calendar days. Falciparum malaria is also a tertian malaria, although the interval may be less than 48 hours, giving rise to the sometimes-applied term *subtertian malaria*. Malaria caused by *P. malariae* is called *quartan malaria*, the episodes occurring every 72 hours. Vivax malaria is furthermore called *benign tertian malaria* and falciparum malaria *malignant tertian*, reflecting the difference in the severity of the disease caused by the two.

In a malaria attack the patient initially experiences chills for 15–60 minutes coincident with the release of a brood of merozoites. This is usually accompanied by headache, nausea, and vomiting. Succeeding the chills is a febrile stage of several hours' duration. Temperatures sometimes spike to 105°F or higher. Profuse sweating concludes the paroxysm. The exhausting episode induces sleep, from which the patient awakens with a feeling of comparative well-being. The infections may be asymptomatic, severely enfeebling, or fatal. When the red blood cells rupture, malarial pigments, hemoglobin, and metabolites of the parasite are also released. These products are taken up especially by the RES and account for the marked pigmentation and enlargement seen in the spleen, liver, lymph nodes, and bone marrow. The parasitized red blood cells become sticky and plug up the smaller vessels, especially in falciparum malaria. The obstructing action of the "sludge blood" has a variety of effects: tissue anoxia (lack of oxygen) and necrosis, bursting of vessels, electrolyte imbalance, and other vessel-associated pathology. Many organs may be affected. Some of the more dramatic pathological changes associated with malaria occur in the brain and the kidneys.

LABORATORY DIAGNOSIS. The definitive diagnosis of malaria rests on discovery of the typical parasite forms in stained blood smears. Thick and thin blood smears are prepared: The drop of blood is spread out into a film one cell thick for the thin blood smear, whereas for the thick smear drops of blood are only slightly spread out. The thick smear allows for a greater concentration of the parasites so that an infection is not readily overlooked. The red blood cells of the thick smear are laked (lysed, dehemoglobinized), thereby eliminating intact red blood cells that make it difficult for the inexperienced viewer to discern the malarial forms. The experienced malariologist readily recognizes the malarial forms and is furthermore able to differentiate the plasmodial species because of differences in trophozoite forms, malarial pigment, numbers of merozoites, character of the infected red blood cells, and other distinguishing features.

TREATMENT AND CONTROL. Chemotherapy for malaria is complex. It may be applied for prophylaxis, for the suppression or prevention of an attack in an infected individual, and for cure. Two mainstays of malaria chemotherapy are chloroquine, a 4-aminoquinoline (used for the erythrocytic stage), and primaquine phosphate, an 8-aminoquinoline (used for the exoerythrocytic stage). It is necessary to eliminate the tissue phase in malaria types in which the parasite persists in the liver because the plasmodia that survive in the liver can reestablish clinical malaria. *P. malariae* infections of 40 years' persistence have been reported. Drug-resistant strains, notably strains of *P. falciparum* that are resistant to chloroquine and other antimalarials, pose continuing challenges in malaria control. Chloroquine, if it were not for the development of resistance against it, is a model antimalarial because of its low cost, low toxicity even for neonates and pregnant women, long half-life, and rapid action against the erythrocytic phase of the parasite (i.e., against the

schizonts). Alternative drugs have a variety of shortcomings. The combination of sulfadiazine plus pyrimethamine, for example, which became licensed in the United States in 1982, is not recommended for those with sulfa allergies, nor for pregnant women because of teratogenicity, nor for infants below the age of 2 because it causes jaundice. Furthermore, plasmodial resistance to this combination is already developing.

Considerable efforts are being made in a network of more than 30 malaria research centers to develop an antimalaria vaccine. A successful vaccine would do much to reduce the immensity of the malaria control problems, which include the parasites' resistance to chemotherapeutic agents, the resistance of mosquito vectors to insecticides, and the cost of current control measures. The ideal vaccine would contain antigens that stimulate protection against the three life stages of plasmodia—sporozoites, merozoites, and gametocytes. Surface antigens of the plasmodia are currently being cloned for use in vaccines. A sporozoite vaccine has been demonstrated to provide immunity in animals.

Mosquito control measures include the destruction of mosquito larval forms, the elimination of mosquito breeding places, netting, and repellents.

The development of resistance to insecticides by mosquitoes and the persistence of insecticide residues in the environment, along with the resistance of the malaria parasite to drugs, were major difficulties that caused an indefinite postponement of the WHO plan to eradicate malaria.

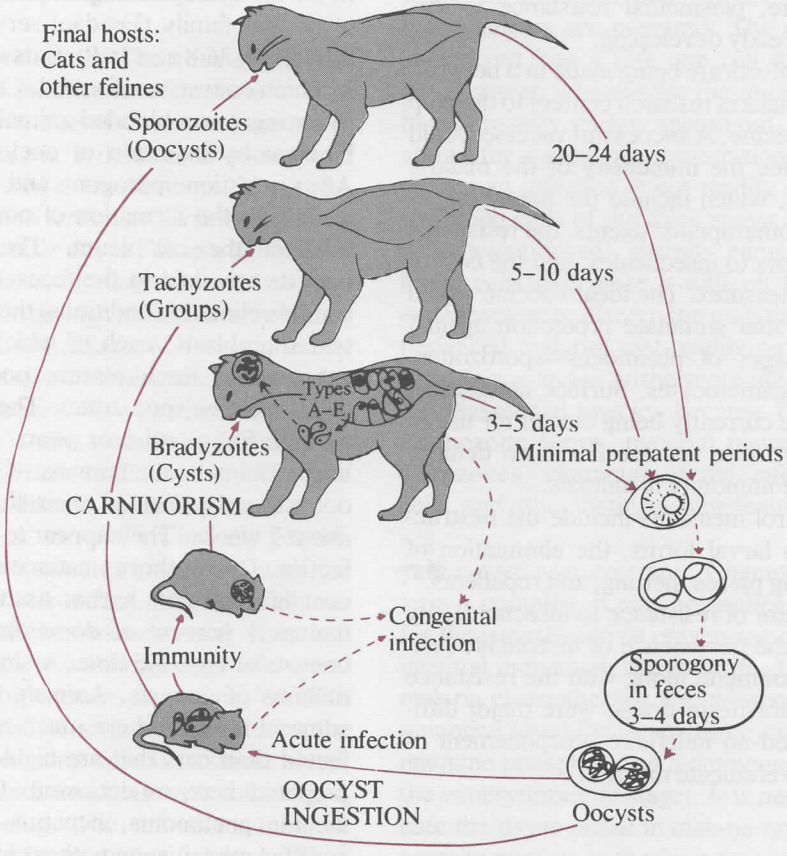
Toxoplasmosis

Toxoplasma gondii infects a wide range of mammals and birds. Infection of humans is common, ranging from 5 to 80 percent in various populations throughout the world. The organism occurs in all hosts as tachyzoites (rapidly dividing forms) that accumulate in host cells and as bradyzoites (slowly dividing forms) that accumulate within a parasitic membrane called a cyst. Tachyzoites are often crescent-shaped or bow-shaped (*toxon* = bow or arc) organisms, 4 to 8 μm in length and 2 to 3 μm in width, with the anterior end pointed and the other end rounded or blunted. The bradyzoite-containing cysts are from 5 μm to 1000 μm in size and may house hundreds of parasites. The cysts are found mainly in the brain, eye, and muscles where usually they persist harmlessly after the host has acquired immunity.

TOXOPLASMA GONDII IN MEMBERS OF THE CAT FAMILY. In the life cycle of *Toxoplasma* organisms members of the cat family (Felidae) serve as the definitive host (Figs. 36-8 and 36-9). Cats are the only animals in which oocysts are formed. Cats become infected by eating warm-blooded animals such as mice and birds or by ingestion of oocysts from cat feces. After ingestion sporogony and gametogony occur, leading to the formation of oocysts in the epithelium of the cat ileum. The zygote-containing oocysts are shed in the feces. In 3–4 days (under suitable climatic conditions) the zygote divides into two sporoblasts, each of which gives rise to four sporozoites. Each mature oocyst thus contains eight infective sporozoites. The mature oocyst can survive for months or years in nature, there to infect animals or humans. Cats probably shed oocysts only once in their lives for a period of about 2 weeks. They appear to be resistant to reinfection. One authority estimated that 30 to 60 percent of adult cats harbor tissue cysts and that as few as 1 percent of domestic cats are shedding oocysts at any one time. A single cat may excrete millions of oocysts. Animals that hunt for all or some of their food are much more likely to be infected than cats that are highly domesticated (eat prepared diets, are declawed). Overt disease in cats include pneumonia, hepatitis, pancreatitis, myocarditis, myositis, and other clinical conditions that result from the inflammation or necrotization of affected tissues. Congenitally acquired infection probably does not occur.

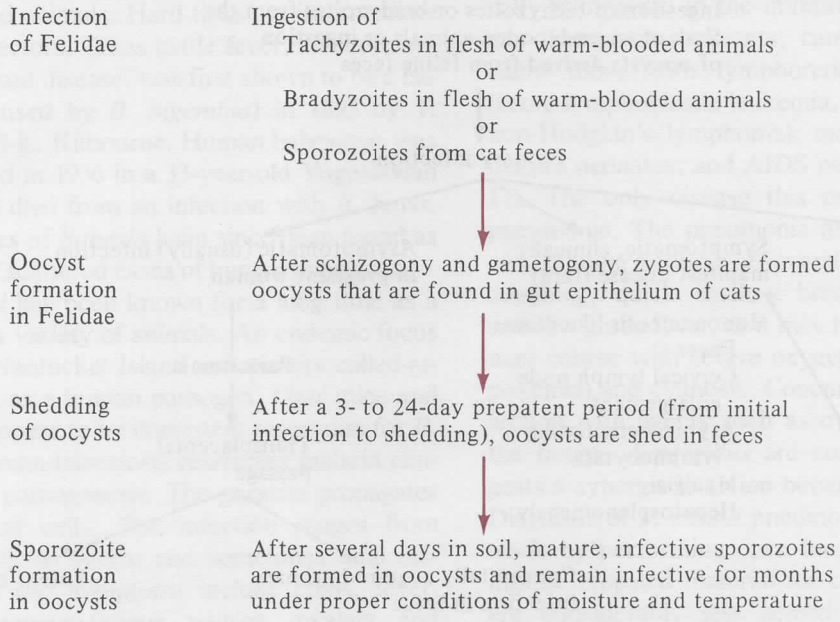
TOXOPLASMA GONDII INFECTIONS IN HUMANS. Human infection (Fig. 36-10) is believed to stem primarily from the ingestion of raw or undercooked meat. Ingestion of oocysts is another route. Swine, sheep, and rabbits throughout the world are often infected. How herbivorous animals become infected is not known. Initial infection commonly leads to parasitemia.

Fig. 36-8. Life cycle of *Toxoplasma*. Three cats are used to indicate infections with different prepatent periods (period between ingestion of organism to oocyst appearance). After the ingestion of bradyzoites (cysts), the minimal prepatent period is 3–5 days. After the ingestion of tachyzoites of the acute infection, the minimal prepatent period is 5–10 days. After the ingestion of sporozoites (oocysts), the minimal prepatent period is 20–24 days. (From J. K. Frenkel, Toxoplasmosis: Parasite Life Cycle Pathology, and Immunology. In D. M. Hammond and P. L. Long [Eds.], *The Coccidia*. Baltimore: University Park Press, 1973.)



The most common outcome of primary infection is asymptomatic infection. A very small percentage of primarily infected individuals develop clinical disease, which is usually mild and rarely fatal. Most often an overt primary infection resembles mononucleosis: enlarged cervical lymph nodes, hepatosplenomegaly, myalgia, and atypical lymphocytes. Tissue cysts develop in various organs after primary infection and are accompanied by immunity. The tissue cysts are dormant (latent) and are probably of no further consequence except when reactivation occurs in immunocompromised transplant recipients, in those who are immunosuppressed because of cancer therapy, in victims of acquired immune deficiency syndrome (AIDS), and in those whose cancer affects the lymphatic system. Reactivation can lead to disseminated toxoplasmosis that can be fatal. Disseminated infection

Fig. 36-9. *Toxoplasma gondii* cycle in the definitive host. (Felidae = cat family.)

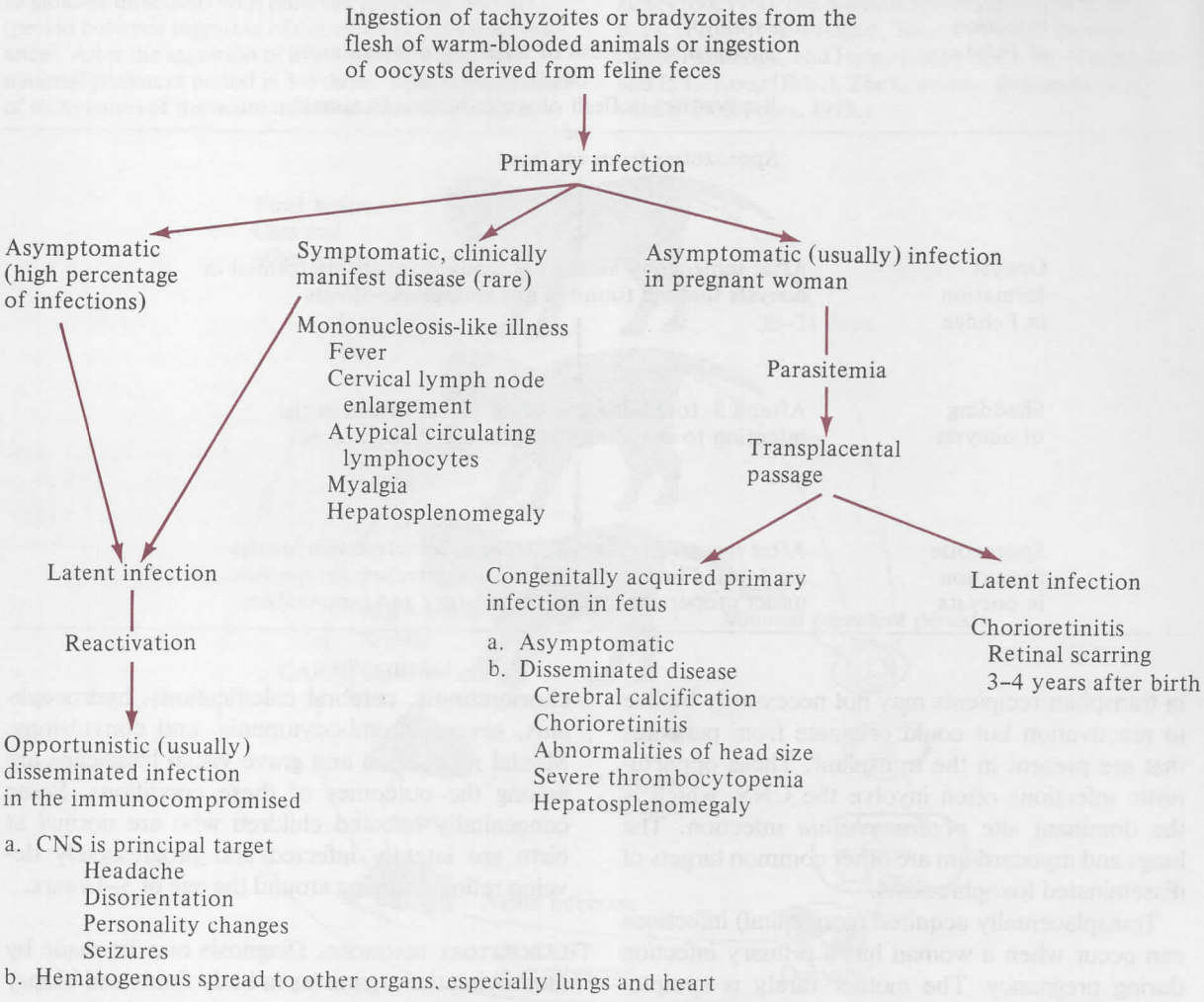


in transplant recipients may not necessarily be due to reactivation but could originate from parasites that are present in the transplant. These opportunistic infections often involve the CNS, which is the dominant site of *Toxoplasma* infection. The lungs and myocardium are other common targets of disseminated toxoplasmosis.

Transplacentally acquired (congenital) infections can occur when a woman has a primary infection during pregnancy. The mother rarely is symptomatic but in the period when she has parasitemia, *Toxoplasma*-containing cysts may form in the placenta, where the parasite causes a generalized infection of the fetus. Infection of the fetus, according to one study, occurs in about 50 percent of cases in which the mother has a primary infection during pregnancy. A high percentage of children that were congenitally infected have no disease sequelae and remain well. It is estimated that less than 1 percent are born with clinical disease. A wide range of illness results from congenitally acquired toxoplasmosis. The seriously diseased have chorioretinitis, cerebral calcifications, hydrocephalus, severe thrombocytopenia, and convulsions. Mental retardation and grave visual handicaps are among the outcomes of these conditions. Some congenitally infected children who are normal at birth are latently infected and progressively develop retinal scarring around the age of 3–4 years.

LABORATORY DIAGNOSIS. Diagnosis may be made by identifying the organisms in body fluids and biopsy tissues or by infecting mice with material from appropriate specimens. Diagnosis is usually based on serological tests, however. The serological test specifically used in *Toxoplasma* infection is the Sabin-Feldman dye test, in which anti-*Toxoplasma* antibodies in the patient's serum prevent the uptake of methylene blue dye by living *Toxoplasma* test organisms. The living organisms are stained by the dye in the absence of specific antibodies. Other serological tests include direct and indirect fluorescent antibody, complement fixation, and enzyme-linked immunosorbent assay (ELISA).

Fig. 36-10. *Toxoplasma gondii* infections in humans (an intermediate host). (CNS = central nervous system.)



TREATMENT AND CONTROL. The drugs of choice are trisulfapyrimidines (combinations of sulfadiazine, sulfamerazine, and sulfamethazine) plus pyrimethamine. Spiramycin is an alternate drug that is especially prescribed during pregnancy to avoid the teratogenic effect of sulfadiazine and pyrimethamine.

Practical recommendations based on knowledge about the epidemiological characteristics of *Toxoplasma* can help control the disease and are especially pertinent for pregnant women. The recommendations include careful handling of raw meat and the heating of meat to 150°F throughout, daily cleaning of cat litter boxes, disinfection of litter pans with boiling water, covering of sandboxes when not in use, and wearing gloves while gardening.

Babesiosis (Nantucket Fever)

There are 71 species of *Babesia* that infect wild and domesticated animals. Hard ticks are intermediate hosts and vectors. Texas cattle fever, an economically important disease, was first shown to be a babesiosis (caused by *B. bigemina*) in 1893 by T. Smith and F. L. Kilbourne. Human babesiosis was first reported in 1956 in a 33-year-old Yugoslavian farmer who died from an infection with *B. bovis*. Other species of *Babesia* have since been found as the cause of scattered cases of human babesiosis.

B. microti has been known for a long time as a parasite of a variety of animals. An endemic focus in 1975 on Nantucket Island and vicinity called attention to it as a human pathogen. Deer mice and field mice appear to be consistent reservoirs for *B. microti*. Human babesiosis resembles malaria clinically in its pathogenesis. The parasite propagates in red blood cells. The infection ranges from asymptomatic to severe and sometimes fatal disease. Signs and symptoms include chills, fever, drenching sweats, fatigue, nausea, myalgia, and emotional changes.

Laboratory diagnostic procedures include the examination of stained thick and thin blood films from the patient or from hamsters and gerbils that have been inoculated intraperitoneally with patient's blood, and serological testing, especially an indirect fluorescent antibody test. Microscopically, *B. microti* is a 1.0- to 5.0-μm pear-shaped, round, or oval organism that at certain stages resembles *P. falciparum* (the malaria organism). An occasional intraerythrocyte arrangement peculiar to babesiae is the orientation of four (tetrad) trophozoites in a formation that resembles the Maltese cross. Specific, effective chemotherapy has not been determined. Drugs that have been used include chloroquine, pentamidine isethionate, and a combination of clindamycin and quinine.

Pneumocystosis

Animal studies indicate that *Pneumocystis carinii*, a sporozoon of cosmopolitan distribution, is present in the lungs of most humans. It evidently infects many kinds of wild and domestic animals. Its life cycle and mode of transmisstion (inhaled cysts?) are not clearly known. *P. carinii* is opportunistically pathogenic in the immunocompromised: organ transplant recipients; cancer patients, especially those with lymphoreticular malignancies (acute lymphoblastic leukemia, Hodgkin's disease, non-Hodgkin's lymphoma); malnourished or premature neonates; and AIDS patients (see Chapter 17). The only disease this protozoan causes is pneumonia. The pneumonia may either be insidious, running a course of several days to weeks with coughing, quick shallow breathing (tachypnea), and low-grade fever, or it may have an acute fulminant course with severe oxygen insufficiency (hypoxemia) and cyanosis. Concurrent pulmonary infection with agents such as cytomegalovirus and the fungus *Aspergillus* are common, which suggests a synergistic action between two organisms. Diagnosis of *P. carinii* pneumonia is most reliably made by performing open lung biopsy and staining the biopsied material for cysts. Methods that are considerably less sensitive because of the limited size of the sample obtained for staining include transtracheal aspiration, bronchopulmonary lavage, endobronchial brush biopsy, transbronchial biopsy, and percutaneous fine needle aspiration. The individual organisms observed microscopically are 5- to 10-μm oval bodies that may resemble overinflated footballs in imprinted smears, or when sectioned on edge may appear as helmets or sickles. Comma-shaped bodies, often paired, can be seen in the cysts. Cultivation presently is difficult and requires a suitable specimen. Serological tests so far are not as reliable as microscopical observations. Sulfamethoxazole-trimethoprim chemotherapy is effective when provided early.

Cryptosporidum Species—Cryptosporidiosis

Cryptosporidium species belong to a group of protozoan parasites called Coccidia, which also includes *Toxoplasma* and *Pneumocystis*. Until recently cryptosporidiosis was considered a rare animal disease that was even more rarely transmitted to humans. *Cryptosporidium* species are now well known to veterinarians because they are im-

portant causes of gastroenteritis and diarrhea in animals.

Cryptosporidiosis is transmitted to humans by oocysts that have been passed in the feces of farm animals and probably by most domestic animals. Human to human transmission is highly probable by direct or indirect contact with contaminated feces or by contact with respiratory secretions.

Cryptosporidiosis causes fatal intractable watery diarrhea in compromised individuals, but a self-limited enteritis in normal hosts. Since 1980 several cases of cryptosporidiosis (over 50) have been observed in patients with AIDS as well as those undergoing immunosuppressive therapy. The average fluid loss in immune deficient patients is about 3 to 6 liters per day but as much as 17 liters per day has been reported.

The oocysts of *Cryptosporidium* species are no longer than yeasts (4 to 6 μm) and go undetected in fecal specimens unless special techniques are utilized. Currently the Ziehl-Neelsen acid fast technique is used to detect and differentiate this protozoa from yeast. The oocysts are acid-fast but yeasts are not acid-fast. Fluorescent antibody techniques can also be applied to stool preparations to detect oocysts. *Cryptosporidium* can also be found in the pharynx, esophagus, and stomach as well as the upper and lower intestine of immunodeficient patients.

The prognosis for immunocompromised patients with cryptosporidiosis is poor since a variety of therapies have proved unsuccessful. Treatment is not necessary for immunocompetent individuals infected with the parasite.

THE NEMATHELMINTHES

Members of the animal phylum Nemathelminthes are referred to as the *roundworms* because they are round or oval in cross section. Their body form is elongated and cylindrical, tapering at both ends. Unlike the earthworm, they are nonsegmented. They have a well-developed digestive tract. The sexes are separate (the organisms are dioecious).

ASCARIASIS (ROUNDWORM INFECTION)

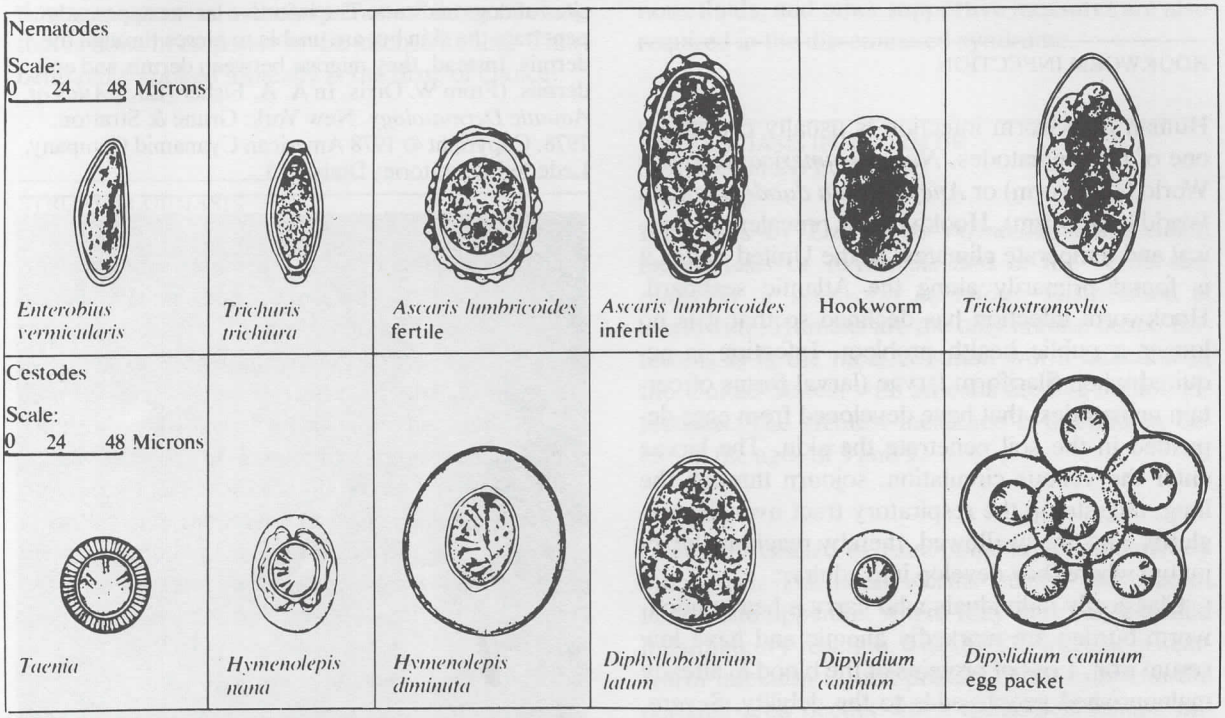
Ascaris lumbricoides is the largest intestinal nematode to infect humans. The adult worms are 8 to 12 inches long. They resemble the earthworm in size and overall configuration. The males, which are smaller than the females, have an incurved tail.

A. lumbricoides is found worldwide. It causes one of the most prevalent parasitic infections of humans; virtually all members of the population in some areas of the world harbor the parasite. Fecal contamination and the use of human excreta as fertilizer are mainly responsible for the maintenance of the parasite in regions where ascariasis is endemic. Infective larvae develop in about 2–3 weeks in fertilized eggs that have been deposited in the soil. The eggs remain viable for months, possibly as long as 10 years. Immigrants from areas where ascariasis is highly endemic do not pose a threat because adequate sewage disposal prevents exposure of the public to the infective eggs.

Symptoms and Pathogenesis

The larvae in ingested eggs hatch out in the duodenum, penetrate the mucosa, enter the blood and lymph vessels, and are transported to the lungs. At this juncture the larvae, if present in large enough numbers, elicit hemorrhage and inflammation to a degree sufficient to cause pneumonitis or an asthma-like response. Since *Ascaris* eggs are not found in the feces at this time, the pneumonitis is difficult to diagnose. From the lungs the larvae move up the respiratory tree, enter the esophagus, and are swallowed. Adult worms develop in the intestine, where they may cause vague abdominal symptoms. Because of their proclivity to probe and meander they may obstruct the excretory ducts of the pancreas or the biliary tract, or they may appear in many other parts of the body, to emerge, for example, in rare horrifying instances from the nares. Heavy infections, more likely to be found in children, may produce toxic symptoms or intestinal obstruction. Combinations of *Ascaris*, *Trichuris*, and hookworm infections in the same individual are not uncommon in areas of hyperendemism. The vitality-sapping parasitic infections, com-

Fig. 36-11. Nematode and cestode eggs found in human stool specimens. (From M. M. Brooke and D. M. Melvin, *Morphology of Diagnostic Stages of Intestinal Parasites of Man*. Atlanta: Public Health Service Publication #1966, Center for Disease Control, 1969.)



pounded by the malnutrition that is ever present in economically depressed areas, may produce physical and mental retardation.

Laboratory Diagnosis

The identification of the typical fertilized or unfertilized eggs in the feces is diagnostic (Fig. 36-11).

Treatment

Mebendazole or pyrantel pamoate are the drugs of choice. Drugs administered for other parasitic infections may cause *Ascaris* worms to curl up. If there is a heavy *Ascaris* worm burden, the curled-up compacted worms may cause intestinal obstruction requiring surgical intervention. There is no anthelmintic (anti-worm) drug that destroys the larvae during the period of lung migration.

Visceral Larva Migrans

Larvae of nematodes that infect other mammalian species sometimes infect humans, but they are unable to develop into adult worms. They survive for a period of time to migrate through organs of the body (visceral larva migrans) or to tunnel through the skin (cutaneous larva migrans; see under Hookworm Infection). In visceral larva migrans (VLM) there may be fever, infiltration of the lungs, hepatomegaly (enlargement of liver), hyperglobulinemia (increased globulin in the blood), and granuloma formation in the brain, eye, lungs, and other organs. VLM is most likely to occur in children between the ages of 1 and 5 who are in contact with pets or who have pica (an unnatural craving for bizarre foods such as dirt). Most often implicated in VLM are dog and cat ascarids (close relatives of *A.*

lumbricoides) in the genus *Toxocara*. *Toxocara canis* is found in at least 20 percent of the 30 million dogs in the United States.

HOOKWORM INFECTION

Human hookworm infection is usually caused by one of two nematodes, *Necator americanus* (New World hookworm) or *Ancylostoma duodenale* (Old World hookworm). Hookworm is prevalent in tropical and temperate climates. In the United States it is found primarily along the Atlantic seaboard. Hookworm infection has declined so that it is no longer a public health problem. Infection is acquired when filariform larvae (larval forms of certain nematodes) that have developed from eggs deposited in the soil penetrate the skin. The larvae enter the venous circulation, sojourn through the lung, migrate up the respiratory tract over the epiglottis, and are swallowed, thereby reaching the jejunum where they develop into adults.

Classically individuals who carry a heavy hookworm burden are markedly anemic and have low serum iron. Loss of proteins of the blood in already malnourished people adds to the debility. Severe, protracted, or repetitive infections, of children especially, may lead to physical stunting and mental retardation.

Laboratory Diagnosis

Routine diagnosis is based on identification of the eggs in feces (see Fig. 36-11). Counts of eggs per unit weight of feces are sometimes performed to estimate the severity of the disease or to determine the effectiveness of a therapeutic measure.

Treatment and Control

Mebendazole is the drug of choice, as it is for most non-tissue-invasive, lumen-dwelling parasites. Pyrantel pamoate is another drug of choice. It is necessary to remedy the malnutrition and the loss of iron in the severely diseased. Blood transfusions may be required in a few isolated cases. Control is difficult where hookworm is hyperendemic because the soil in areas of human habitation is being constantly contaminated with human excreta and the wearing of footwear is not customary. Chemotherapy is, therefore, only temporarily effective and will probably remain so until the population is educated to dispose of human excreta properly.

Fig. 36-12. Creeping eruption in a human being. This dermatosis is caused by larval hookworms of the species *Ancylostoma braziliense, Uncinaria stenocephala*, and perhaps *A. caninum*, all of which are natural parasites of dogs and cats. The infective larvae apparently penetrate the skin but are unable to pierce through the dermis. Instead, they migrate between dermis and epidermis. (From W. Orris. In A. A. Fisher [Ed.], *Atlas of Aquatic Dermatology*. New York: Grune & Stratton, 1978. Copyright © 1978 American Cyanamid Company, Lederle Laboratories Division.)

Cutaneous Larva Migrans

Filariform larvae of dog and cat hookworm species (*Ancylostoma braziliense* and *Ancylostoma canium*) and larvae of nonhuman species of *Strongyloides* penetrate the skin of children, farmers, craftsmen, and others who have contacted warm, moist, sandy soil, especially where dogs and cats have defecated. The larvae burrow into the subcu-

taneous tissues and produce linear, pruritic, papulovesicular lesions (Fig. 36-12). The condition is referred to as *serpiginous dermatitis, creeping eruption*, or *ground itch*. Secondary bacterial infection may occur as a consequence of the scratching that is done in response to the intense itching. Thiabendazole applied topically is the drug of choice.

STRONGYLOIDIASIS

Strongyloides stercoralis is an intestinal parasite of humans. It is found especially in warm, moist, tropical climates. In the United States it is most common in the rural Southeast. Its life cycle and distribution resemble those of the hookworm. There are two larval forms, the rhabditiform (so-called because of a bulb-like prominence in the esophagus) larva and the filariform larva. The filariform larva is the infective (transmission) form. In the usual mode of infection filariform larvae penetrate the skin and pass via the venous circulation to the lungs whence they are coughed up to the level of the epiglottis and subsequently swallowed, thereby gaining entrance to the upper intestinal tract. The female larva burrows into the mucosa, matures, and ovideposits (lays eggs). The ova hatch, liberating rhabditiform larvae that transform into filariform larvae in the intestine, on the perianal skin, or in the shed feces. The three sites of transformation are reflected in the three modes of infection: internal autoinfection through the intestinal mucosa, reinfection through the skin of the perianal area, and (most commonly) contact with feces or fecally contaminated soil. Rhabditiform larvae shed in the feces either transform into filariform larva directly or mature and form a free-living generation in which there are adults that copulate and produce infective larvae. *S. stercoralis* is unusual among nematodes because it has a free-living generation and a parasitic generation.

Most infections are asymptomatic. Heavy infections can cause anorexia, diarrhea, ulceration of the mucosa, and anemia. In internal autoinfection filariform larvae may disseminate throughout the body of a compromised host, leading to a life-threatening situation.

Uncomplicated strongyloidiasis is effectively treated with thiabendazole. Antibiotics, intravenous fluids, and other supportive measures are also required in the disseminated syndrome.

ENTEROBIASIS (PINWORM OR SEATWORM INFECTION)

Infections by *Enterobius vermicularis* are called *enterobiasis* or *oxyuriasis* and in the vernacular *pinworm* or *seatworm infections*. Distribution is worldwide. Humans are the only natural hosts. Enterobiasis is the most common worm infection in the United States, with an estimated 42 million infections. The greatest incidence is in children between the ages of 5 and 9.

Symptoms

Infections caused by *Enterobius* are comparatively innocuous. The adult worms inhabit the cecum, ileum, and appendix where they may cause a mild inflammatory reaction. The chief complaint of seatworm infection is anal pruritus. Discomfort in the perianal area occurs when the gravid female migrates out of the anus, usually during the night, to deposit eggs in the perianal, perineal region. The intense itching encourages scratching, which in turn may abrade the area sufficiently to introduce secondary bacterial infection. Scratching, furthermore, collects eggs under the fingernails and on the fingers, thence to autoinfect or to contaminate articles that will spread the infection to others. The eggs are found on clothing, bedding, windowsills, floors, and elsewhere in the environment of an infected individual.

Laboratory Diagnosis

The transparent tape swab is generally used for diagnosis. Parents are frequently asked to obtain the specimen when the child first awakens in the morning. The sticky side of the tape is applied several times to the anal area. The tape is then fastened, adhesive side down, to a glass slide. The contact

areas are examined under the microscope for the presence of the typical flat-sided eggs (see Fig. 36-11). The same technique is sometimes used in tapeworm infections.

Treatment and Control

Several highly effective and relatively nontoxic drugs can produce a 90 to 100 percent cure rate. Mebendazole and pyrantel pamoate are the drugs of choice. Piperazine citrate and pyrvinium pamoate are alternative drugs. If pyrvinium pamoate is prescribed patients should be told that this drug is a dye that will cause stools and vomitus to appear bright red. The life span of the worm is about 1 month. Reinfection is not uncommon, so retreatment may be necessary. Since enterobiasis is a family infection, all household members should be treated. Scrupulous hygienic practices such as boiling bed linens and disinfecting toilet seats are sometimes recommended to rid a family of the pinworms. It is questionable how effective and how necessary these practices are, especially in view of the psychological trauma such admonitions may initiate in conscientious parents. Rather, parents should be told of the commonness of pinworm infection, its relative innocuousness, and the effectiveness of drug treatment.

TRICHINOSIS (MUSCLE WORM INFECTION)

Trichinosis (trichinelliasis) was one of the more prevalent worm infections in the United States, but it has greatly diminished. Autopsies conducted in the 1930s and early 1940s revealed an incidence of about 16 percent. Recent autopsy surveys show an incidence of about 2 percent. Most infections are subclinical. Since 1970 the reported morbidity has been about 100 cases each year. There has been an average of fewer than two deaths per year in that time.

Life Cycle

A number of animals serve as hosts for *Trichinella spiralis*, the adult and larval forms appearing in the same animal. When flesh that contains live encysted larvae is ingested, the larvae emerge in the duodenum and mature into adults (Fig. 36-13). Impregnated females penetrate the wall of the intestine and start to produce live larvae after about 1 week. Each female produces about 500 larvae over a 2-week period. The shower of larvae enters the capillaries and lymphatics and migrates through the lungs into the heart and the systemic circulation. No tissues are immune to invasion. The heaviest infections are in the striated muscles, but the heart, the CNS, and the serous cavities are also favored sites of localization. The larvae coil up in the muscle fibers, and the body surrounds the area with a calcified wall in 6 months to 2 years. The encysted larvae may remain viable for as long as 10 years. They are at a dead end here unless the flesh is ingested by another host. Rodents are largely responsible for maintaining the infection in nature. A wide variety of carnivores are infected. Humans contract the infection by eating meat that contains viable encysted larvae. The chief reservoir for humans is infected swine, with wild bear meat as another well-recognized source of infection.

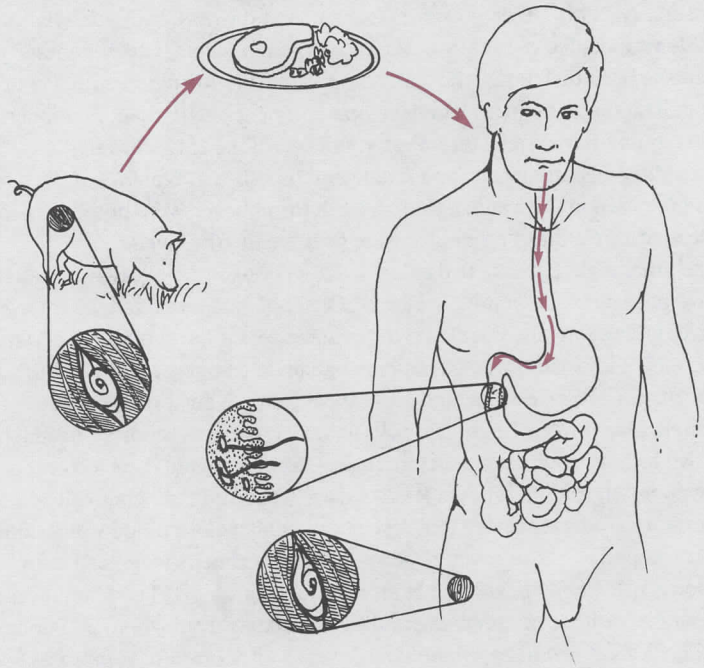
Symptoms and Pathogenesis

The symptoms of trichinosis are related to the clinical phase of the infection. During establishment of the adult worms in the intestine, the symptoms—diarrhea, abdominal discomfort, vomiting—suggest food intoxication or infectious diarrhea. Beginning about the tenth day and reaching a peak in the second to third week when the larvae are migrating and being filtered out in the muscles, periorbital swelling occurs in about 80 percent of symptomatic cases, fever in 90 percent, myalgia in 80 percent, and rash in about 10 percent. It is estimated that at least 100 larvae are required to initiate symptoms. In the third clinical phase, when the larvae are encysted and the body responds with repair measures, the patient experiences muscle pain, weakness, and cachexia (wasting). The symptoms just described are related primarily to muscle invasion, but no tissue or organ is immune.

Laboratory Diagnosis

Trichinelliasis should be suspected in patients who have periorbital edema, fever, and myalgia. The

Fig. 36-13. Life cycle of *Trichinella spiralis*. Infective larvae, encysted in pork and other meat, when ingested, become adult worms in small intestine. The female burrows into the mucosa and deposits larvae in lacteals (intestinal lymphatic vessels that transport absorbed fat) and blood vessels. Circulating larvae eventually penetrate skeletal muscle and become encysted (*A*). In humans these larvae are at a dead end, but in the pig and other animals they become a source of infection (*B*). (From J. W. Beck and J. E. Davis, *Medical Parasitology* [2nd ed.]. St. Louis: Mosby, 1976.)



likelihood of trichinelliasis is increased if others who shared the same diet have the typical signs and symptoms. The surest diagnostic method is detection of the larvae in a muscle biopsy. The biopsy material is taken from a tender swollen muscle. Biopsy is not always necessary if the clinical signs and symptoms are fortified with a positive dietary history, high eosinophilia, and positive serology. The serological tests include bentonite flocculation, complement fixation, fluorescent antibody, and precipitin tests. There is a skin test, but it does not differentiate between past and recent infections.

Treatment and Control

There is no satisfactory treatment for trichinelliasis. Thiabendazole is useful in the very early intestinal phase of the infection, but the infection is rarely diagnosed at this juncture. Treatment is largely for symptomatic relief: salicylates as a mainstay for pain and corticosteroids, with questionable effectiveness, for the inflammation. Human muscle worm infection has declined in the United States because of education of the public, use of deep freezers, and a decrease in the prevalence of infection in swine. The public is repeatedly urged to cook pork thoroughly, to the point at which none of the meat is pink. An internal temperature of at least 137°F kills trichinae. Deep-freezing of meat at a temperature of the average home freezer (5°F or −15°C) for 20 days destroys the trichina larvae in cuts of meat that are less than 6 inches thick. The enactment of a law in 1952 that required garbage fed to hogs to be cooked reduced

COMMON-SOURCE OUTBREAK OF TRICHINOSIS—NEW YORK CITY

Eight cases of trichinosis were reported to the New York City Department of Health from two Brooklyn hospitals on November 16, 1981. The patients were members of three related families of Italian heritage. A ninth case was subsequently diagnosed in the course of the investigation. One patient died. The outbreak appeared to be associated with eating dried homemade pork sausage.

The index patient was a 55-year-old woman who had onset of symptoms on November 4, 1981, 10 days after eating some of the pork sausage. Her initial symptoms included nausea, vomiting, diarrhea, myalgia, and abdominal pain. When she became febrile, she was hospitalized. By the nineteenth day of hospitalization, the patient had developed bronchopneumonia, pulmonary edema, pulmonary artery thrombosis, mild cardiomegaly, chronic myocarditis, hepatomegaly, and renal vein thrombosis. No *Trichinella* larvae were identified in the brain, although fresh thromboses were noted in blood vessels. Multiple muscle samples were positive for *Trichinella*.

The other eight patients had eaten dried, uncooked or lightly fried sausage on two occasions, November 4 and 11. Onset of symptoms ranged from 2 to 13 days after eating the initial serving of sausage. Of the symptoms commonly associated with trichinosis, all patients had fever (ranging from 100.2 to 102.4°F), periorbital edema, and muscle pain. Six of eight patients had abdominal pain; five of eight had diarrhea. Two patients reported headache, and two had subconjunctival hemorrhages. Most patients received oral or intravenous steroid therapy; at least two also received thiabendazole. Laboratory studies revealed that all eight patients whose blood was examined had eosinophilia. Six of seven patients whose specimens were tested for antibody to *Trichinella* with the bentonite flocculation (BF) test had titers compatible with recent infection, and three patients from whom additional specimens were tested approximately 7 weeks after onset of symptoms had a $\geq$ fourfold rise in titer to *Trichinella*.

Interviews with family members revealed that the sausage had been prepared from a pig purchased and slaughtered at a farm in Green County, New York. Two other pigs kept at the farm over the past year had been slaughtered for consumption by the farm owner. The carcass of the pig that had been purchased was brought to New York City where it was hung and dried indoors for 2 days; the carcass was then butchered and sausages were prepared and hung to dry another 10 days. The sausages were then eaten raw by eight patients. The ninth fried the sausage lightly before eating it. Samples of sausage were examined by the hospital pathologist who identified multiple encysted larvae of *T. spiralis*.

Sausage prepared by the farmer with meat from the other two pigs was examined and found to contain *Trichinella*. The farmer insisted that the pigs had been fed only grains. However, the farmer went hunting frequently and fed portions of killed game to pet dogs. It was not known whether the pigs had also eaten portions of such meat.

From *MMWR* 31:161, 1982.

the incidence of *Trichinella* infections in swine from between 5 and 10 percent to 0.3 percent in garbage-fed hogs. The incidence in grain-fed swine has decreased from the former 1 percent to 0.1 percent.

About 70 percent of pork products are processed through federally inspected meat-processing firms. The meat, however, is inspected only macroscopically since microscopical examination is impractical in the automated American abattoirs. Most infections appear to be traceable to homemade sausages and to small, local meat firms (see boxed essay, p. 852). Infected pork may adulterate other types of meats if it is mixed with them, as in hamburger or through the use of a common meat grinder. Smoking, heavy spicing, salting, pickling, and drying of meats do not reliably destroy trichinae.

FILARIASIS

Seven species of roundworms are categorized as filariform worms. They constitute a major health problem in many areas of the world. None, however, is endemic in the United States. Filariform worms are transmitted by bloodsucking arthropods such as mosquitoes, midges, blackflies, and deerflies. The adult worms preferentially inhabit certain tissues in humans, who are their principal definitive hosts. *Wuchereria bancrofti* and *Brugia malayi* parasitize the lymphatics. *Onchocerca volvulus* and *Acanthocheilonema streptocerca* are found in tumor-like subcutaneous nodules, usually at bony protuberances or in the deeper connective tissue whence the larvae frequently emigrate to congregate in the eye, thus leading to blindness or disturbances of vision. *Loa loa*, the "eye worm," migrates throughout the connective tissue, often passing across the cornea. *Mansonella ozzardi* and *Acanthocheilonema perstans* are found in the body cavities, such as the pericardial, pleural, and peritoneal cavities.

Female filariform worms produce live larvae called *microfilariae*. These are ingested by the bloodsucking insects and transformed into infective larvae that are transmitted to the definitive host.

Symptoms and Pathogenesis

In general usage, *filariasis*, unless otherwise qualified, refers to Bancroft's filariasis and filariasis malayi. It is an insidious disease that takes years to develop. Repeated bites of mosquitoes over many years are necessary to build up a population of adult worms to produce the pathology. The adult parasites localize in the lymph nodes and lymph vessels, eliciting host cell infiltration and the proliferation of the endothelial cells of the lymphatics. Eventually the worms die and the affected area is patched over by fibroblasts, as in scar formation. Obstruction of lymph channels leads to lymphedema (accumulation of lymph fluid in the tissue). When this is extensive, grotesque enlargement of limbs or of the external genitalia occurs. The descriptive term *elephantiasis* is applied to the condition. Short-term visitors to areas where filariasis is endemic do not experience such consequences, as World War II military personnel sometimes feared.

Laboratory Diagnosis

Microfilariae are sought for conclusive evidence in the blood of individuals who have a geographical history of residence in endemic areas. The microfilariae are not regularly present in the blood. Blood specimens are customarily drawn at around midnight because of the nocturnal periodicity of the microfilariae. They exit from the deep tissues into the peripheral blood during the hours that the arthropod vector habitually takes a blood meal.

Treatment

The administration of diethylcarbamazine citrate reduces the number of microfilariae in the blood and appears to have some effect on the adults in the tissues. Chronic obstruction in the less advanced stages is sometimes improved by surgery.

THE PLATYHELMINTHES

Members of the animal phylum Platyhelminthes collectively are termed the *flatworms*. They are

characteristically flat when viewed in dorsoventral section. They are bilaterally symmetrical. Nearly all are hermaphroditic (monoecious). The medically important flatworms appear in two classes, the Trematoda or flukes, and the Cestoidea or tapeworms.

TREMATODA—FLUKES

Flatworms of the class Trematoda are referred to as *flukes*. Discussion of the medically important flukes is generally organized on the basis of the habitat of the adult worm in the host. Thus *Fasciola hepatica, Clonorchis sinensis, Opisthorchis felineus*, and *Opisthorchis viverrini* are the liver flukes; *Fasciolopsis buski* is the intestinal fluke; *Paragonimus westermani* is the lung fluke; and species of the genus *Schistosoma* are the blood flukes.

The liver, intestinal, and lung flukes are leaf-shaped, hermaphroditic worms that attach to the host via ventral suckers. Their life cycles are among the most complex found in animal species. Specific species of snails (always) and crustacea (sometimes) serve as intermediate hosts for the flukes' several larval forms (such as miracidia, cercariae, and metacercariae). *F. hepatica*, which infects ruminants primarily, is cosmopolitan. It is the only one of the flukes that is enzootic in the United States, but human infection is rarely reported in the United States. The other hermaphroditic flukes are found mainly in the Orient, where millions of persons are infected. Infections are transmitted to humans largely through the ingestion of raw or poorly cooked fish, crabs, crayfish, and aquatic plants. The symptoms are related to the site of infection. Clinical diagnosis is guided by the anatomical location of the symptoms and by the dietary and geographical history of the patient. Definitive laboratory diagnosis is made through the identification of the typical operculated (having a lid) eggs.

The blood flukes are an exception in that they are not hermaphroditic and the body configuration of the adults is not leaf-like. They produce a major parasitic disease of humans, *schistosomiasis* or *bilharziasis*.

Schistosomiasis (Bilharziasis)

LIFE CYCLE. Schistosome eggs, discharged in the feces or urine, hatch into ciliated miracidia. The miracidium must enter a specific snail species to continue its development. Hundreds of thousands of the next larval form, the free-swimming cercariae, develop and emerge from the snail. Upon contacting human skin, they penetrate within seconds to minutes, to migrate through the lungs to the hepatoportal system. After maturing and mating in the blood vessels of the hepatoportal system, the adults settle in the mesenteric or vesical venules. The adults themselves do not multiply, so the number of adults is determined by the number of cercariae that entered the host. Blood fluke infections may persist as long as 25 years. One explanation for the persistence, as discovered in *Schistosoma mansoni* infections, is that adult blood flukes evade the host's immune system by camouflaging their surfaces with host proteins (serum proteins, blood group antigens, immunoglobulins), thereby preventing recognition by the host's immune system.

PATHOGENESIS. Pathology is due to a large extent to the masses of eggs (300 to 3000 per day per female, depending on the *Schistosoma* species) produced. Hypersensitivity reactions also account for some of the pathology. Less than 50 percent of the eggs are shed in the feces or urine. The remaining eggs are found in the tissues or move along the blood vessels until they become lodged in veins of a smaller caliber. The eggs have protruding spines and knobs that abet such lodgment. The blood vessels and tissues react to the entrapped or embedded eggs with a marked granulomatous reaction and tissue destruction. The liver and spleen enlarge, with esophageal varices (enlarged and tortuous vessels) as an occasional complication. *S. haematobium* may cause urinary tract disease in the form of fibrosis, strictures, or stones. Chronic disease has been implicated as a factor in the development of urinary bladder malignancies. In addition to the classic infections just described, schistosomes cause der-

matitis and acute schistosome infections (Katayama fever).

GEOGRAPHICAL DISTRIBUTION. The definite geographical distribution of the schistosomes is related to the geographical presence of particular species of snails. There has to be a more-than-casual contact with fresh water for a sufficient number of cercariae to penetrate the skin. Cases of schistosomiasis are seen in the United States in persons who have traveled to the areas where the disease is endemic and especially in immigrants from Puerto Rico and the Philippines (see boxed essay, p. 856). The absence of the specific species of snails in the continental United States prevents the completion of the parasite's life cycle.

LABORATORY DIAGNOSIS. In most cases diagnosis is made by the detection of the characteristic eggs in feces or urine. Rectal and bladder biopsies, skin testing, and serological tests may also be valuable.

TREATMENT. Antimony-containing drugs were used for many years, but they have been replaced by drugs that are more effective and less toxic: metrifonate for *S. haematobium*, praziquantel for *S. japonicum*, and oxamniquine for *S. mansoni*.

SCHISTOSOME DERMATITIS. In a schistosomal disease entity that occurs commonly in the United States, humans serve as the accidental hosts to the cercariae of blood flukes of birds and mammals. The condition is called *schistosome dermatitis, swimmers' itch, clam diggers' itch*, or *sea bathers' itch*. When infected birds (especially migratory fowl) and mammals pollute lakes, ponds, and streams, the miracidia that emerge from the schistosome eggs infect snails. The free-swimming furcocercous (fork-tailed) cercariae that emerge from the snails penetrate the skin of humans, lose their tails, and become schistosomulae (juvenile worms). The parasite is at a dead end because maturation of animal schistosomes does not occur in humans. The larval infestation of skin rarely leads to a serious condition. The erythematous pustular lesions (accompanied by intense itching) appear within hours of exposure and subside gradually after the second or third day (Fig. 36-14). Antihistamines and antipruritics may alleviate discomfort.

Fig. 36-14. Cercarial (schistosome) dermatitis. The eruption appeared on the arms of a patient exposed to avian schistosome cercariae while he was sitting on a dock jutting out over a lake. (From H. D. Blankespoor. In A. A. Fisher [Ed.], *Atlas of Aquatic Dermatology*. New York: Grune & Stratton, 1978. Copyright © 1978 American Cyanamid Company, Lederle Laboratories Division.)

CESTOIDEA—TAPEWORMS

Members of the other class of flatworms that contains worms of medical importance, the Cestoidea, are dorsoventrally flat, ribbon-like worms collectively referred to as *tapeworms*. Tapeworm infections occur less commonly and their pathogenic potential is considerably less than roundworm or trematode (fluke) infections.

SCHISTOSOMIASIS AMONG RIVER RAFTERS—ETHIOPIA

On October 20, 1982, 4 days after returning from a rafting trip down the Omo River in Ethiopia, a Colorado resident noted the onset of a low-grade intermittent fever and myalgia. His symptoms persisted but he did not seek medical attention. However, in early February 1983, a call from another rafter, in whom schistosomiasis had just been diagnosed, prompted him to call his physician. Stool examination revealed eggs of *Schistosoma mansoni*, and his serum was positive by the indirect immunofluorescent (IIF) test for schistosomiasis.

CDC and the Colorado Department of Health were independently informed of several other possible cases of acute schistosomiasis mansoni among other participants. Three tour guides had schistosomiasis mansoni diagnosed by stool examination after the trip, and they were symptomatic with intermittent fever, cough, lethargy, and myalgia associated with an absolute eosinophilia ($\geq 600/mm^3$). A fourth guide had developed a nontender, firm mass 1 cm in diameter on her left buttock but had an absolute eosinophilia count of $408/mm^3$ and was otherwise asymptomatic. Her stool sample revealed eggs of *S. mansoni*. The mass was not biopsied but resolved 6 days after treatment with praziquantel.

There were two separate raft trips: the first from September 19 to October 16, and the second from October 27 to December 4. Both followed the same route down the Omo River as another trip that led to an outbreak of schistosomiasis a year earlier. Participants had been made aware of the possibility of contracting schistosomiasis by the tour organization. Thirty people participated in one or both trips, and all four United States–based river guides were on both trips. Attempts were made to contact all participants except five staff from the Ethiopian office. Four non–U.S. citizens and one U.S. citizen could not be located. Of 20 participants contacted, 19 had stool and serological examination performed and completed a questionnaire concerning exposure; one with a positive IIF test but four negative stool examinations and a negative rectal biopsy is excluded from further analysis. Seven (39 percent) of the 18 participants had *S. mansoni* infection confirmed by stool examinations, and 11 (61 percent) were negative on testing three or more stools and had negative IIF tests.

All seven infected and two uninfected rafters developed an illness during or after the raft trip. Six of the seven infected persons had onset of a febrile illness, with eosinophilia consistent with acute schistosomiasis, within 3 weeks after a trip. The seventh was the guide who revealed relatively light infections with 26, 24, and 18 eggs per gram.

No specific site of exposure could be identified. Most participants took few or no precautions other than toweling off, despite an awareness of the risk of acquiring schistosomiasis. However, those who towel-dried most of the time after water exposure had a significantly reduced likelihood of infection; eight of eleven of the noninfected and one of seven of the ill towel-dried after water exposure during the last third of either trip.

From *MMWR* 32:585, 1983.

Fig. 36-15. Important stages and diagnostic characteristics of the tapeworms that commonly parasitize humans. (From E. C. Faust, P. C. Beaver, and R. C. Jung, *Animal Agents and Vectors of Human Disease* [4th ed.]. Philadelphia: Lea & Febiger, 1975.) (Original, Faust).

	Taenia saginata	*Taenia solium*	*Hymenolepis nana*	*Hymenolepis diminuta*	*Dipylidium caninum*	*Diphyllobothrium latum*
Scolex						
Gravid proglottid						
Egg (×200)						
Larval stage(s)						
Intermediate host(s)	Cattle	Hogs, Humans	Direct cycle: Humans Indirect cycle: Fleas, Beetles	Rodents, Fleas, Beetles	Dogs or Cats Fleas	I. *Diaptomus cyclops* II. Freshwater fish

The ribbon-like tapeworms can be divided into regions. The head or *scolex* is a pinhead-size structure that anchors in the mucosa via a holdfast organ. The holdfast structures are cup-shaped suckers or suctorial grooves (bothria) or hooks. The latter emanate from a raised, crown-like structure, the *rostellum*. The head tapers into the narrow neck region, which is the proliferative zone. Segments called *proglottids* proliferate from the neck zone and are known collectively as the *strobila*. Some tapeworms consist of only several segments; others, such as the fish tapeworms, may reach extraordinary lengths of 3000 to 4000 segments. As long as the head region with the proliferative neck region remains attached, new segments will continue to be formed. The series of proglottids progresses from immature proglottids immediately behind the neck region through sexually mature proglottids to the gravid (egg-laden) proglottids at the distal end of the worm. The proglottids contain both male and female reproductive organs. There are no digestive, skeletal, circulatory, or respiratory organs. Niclosamide is the drug of choice for all tapeworms that are in the adult stage.

Six tapeworms cause disease in humans (Fig. 36-15): (1) *Taenia saginata*, the beef tapeworm; (2)

Taenia solium, the pork tapeworm; (3) *Diphyllobothrium latum*, the fish tapeworm; (4) *Hymenolepis nana*, the dwarf tapeworm; (5) *Hymenolepis diminuta*, the rat tapeworm; and (6) *Dipylidium caninum*, the dog tapeworm.

Beef Tapeworm and Pork Tapeworm Infection

Infections with adult beef and pork tapeworms (taeniasis) are seldom of themselves serious. Symptoms, if present, usually take the form of epigastric pain or vague abdominal discomfort. Systemic manifestations are restlessness, dizziness, and convulsions. The principal danger to humans occurs when they become the intermediate hosts to the larval form, the cysticercus larva, commonly called the *bladder worm*. Most known cases of cysticercosis in humans are due to the pork tapeworm larva (*Cysticercus cellulosae*). The tissues of infected swine contain the larvae. Ingestion of the larvae in inadequately cooked pork leads to the development of the adult tapeworm in humans. Sometimes, however, mature eggs get into the small intestine of humans and hatch. The eggs may come from an external source such as food, or autoinfection may occur in individuals that have an adult tapeworm infection. The hatched larvae that enter the bloodstream settle primarily in muscle, brain, and eyes. Cysticercosis is a serious health problem in eastern Europe, Thailand, and especially in Mexico. Three percent of autopsies in Mexico show the presence of the larvae. Cysticercosis is the most common parasitic disease worldwide that affects the CNS. Surgical removal of the cysts has been the treatment of choice unless the cysts are located in an inoperable site. Recent clinical trials indicate that praziquantel and metrifonate are effective. Routine applications of these chemotherapeutic agents await additional evaluations.

Diphyllobothriasis

The fish tapeworm (*Diphyllobothrium latum*) is found in temperate climates where the eating of freshwater fish is common—in the Great Lakes regions of the United States and Canada, in Alaska, and in Europe, especially Finland. Infection, which is acquired by ingestion of plerocercoid larvae (See Representative Life Cycles, earlier in this chapter), is usually clinically asymptomatic, but abdominal discomfort, diarrhea, constipation, and, most important, anemia do occur. About 1 percent of infected individuals suffer a macrocytic, hyperchromic anemia.

Cooking fish at a temperature of 56°C (135°F) for 5 minutes or freezing to −18°C (0°F) for 24 hours or to −10°C (14°F) for 72 hours prevents infection. Fish properly brined before smoking should not constitute a source of infection.

THE ARTHROPODA

Arthropods are the most highly organized of the invertebrate animals. Sometime during their life cycle they have paired, jointed appendages, a chitinized (horny polysaccharide) exoskeleton, and a hemocele (body cavity through which a blood-like fluid circulates). The majority of the medically important arthropods are found in the classes Insecta and Arachnida. Flies, fleas, lice, bees, and wasps are members of the class Insecta, arthropods with three pairs of walking legs. Spiders, ticks, and mites are arachnids, arthropods with four pairs of walking legs.

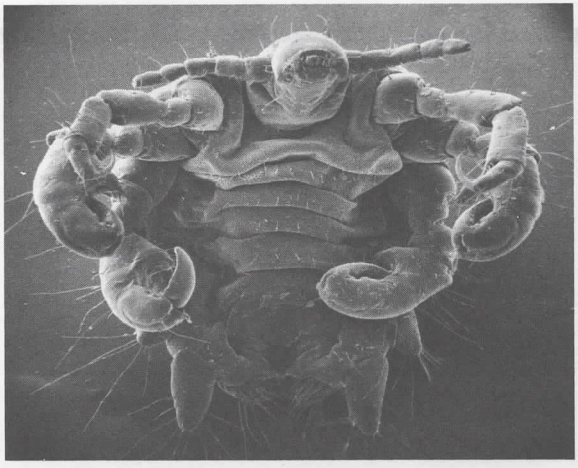
Arthropods periodically shed (molt) the entire exoskeleton. The stages between moltings are called *instars*. Immature instar forms that resemble the adults are called *nymphs*; those that are morphologically quite distinct from the adult form are called *larvae*. Larvae are worm-like in character. In some arthropods there is a nonfeeding form, the pupa, that appears between the larval form and the adult form.

The medical importance of arthropods will only be noted here. The larvae and adults may affect humans in a number of ways: as ectoparasites, as intermediate or definitive hosts of other animal parasites, as venom-producing agents, or as vectors of infectious disease.

As ectoparasites they cause disease while feed-

Fig. 36-16. Forepart of the tick. When biting, the tick thrusts the serrated biting organ (*center*) through the skin into deeper-lying tissues. It is the serrations that make dislodgment so difficult. (From P. B. Armstrong, D. W. Deamer, and J. J. Mais, What's Biting You? *J. Natl. Hist.* June/July 1972.)

Fig. 36-17. A human crab louse from underneath. The thick legs and large claws are used to grasp hair shafts on the host. (From P. B. Armstrong, D. W. Deamer, and J. J. Mais, What's Biting You? *J. Natl. Hist.* June/July 1972.)



ing or taking up abode on the skin and underlying tissues. That association is an *infestation*. Examples of arthropods that cause infestations of humans are the itch mite (which causes scabies) and the larval mites known as *chiggers*; fleas (which have backward-projecting bristles and spines that enable them to proceed through hair and fur without becoming entangled), often from infested pets; ticks (Fig. 36-16), bedbugs, and kissing bugs; the body louse, the head louse, and the pubic or crab louse (Fig. 36-17); and larval forms of flies (maggots) that infest the body (myiasis).

The role of arthropods as intermediate and definitive hosts should have been apparent in the preceding descriptions of the parasitic diseases. As venom producers arthropods such as bees, wasps, spiders, and tarantulas produce substances that are toxic or that induce allergic reactions (see Chapter 17 under The Agents of Human Anaphylaxis). Venenation (use of venom) is not parasitosis; that is, the venom-producing arthropods do not lead a parasitic type of existence. The importance of arthropods as mechanical and biological vectors of bacterial, viral, rickettsial, and mycotic disease agents is evident in the descriptions of those diseases. In summary, arthropods directly cause medical problems as ectoparasites and as venenators, or they do so by fulfilling a role in the development or transmission of other disease agents.

SUMMARY

Traditionally the term *the parasites* is applied to the infectious disease agents that are found in four divisions of the animal kingdom, the Protozoa, the Nemathelminthes (roundworms), the Platyhelminthes (flatworms), and the Arthropoda. The distribution and incidence of parasitic disease is governed by such factors as availability of suitable definitive and intermediate hosts and appropriate vectors, climate, socioeconomic conditions, sanitary practices, and ethnic eating habits. The infective forms that are involved in the transmission of

parasites are the trophozoites, cysts, and sporozoites of protozoa and the eggs and larvae of worms. The major mode of laboratory diagnosis is identification by microscope of the various morphological forms of trophozoites, cysts, ova, and larvae. Control of parasitic disease includes the destruction of vectors and intermediate hosts and management of their breeding places, education of the populace, proper sanitation, and the use of appropriate drugs.

It is not possible to summarize all the parasitic diseases, so the following notes are presented.

AMEBIASIS amebic dysentery, extraintestinal amebiasis, ameboma, cyst passers, night soil

PRIMARY AMEBIC MENINGOENCEPHALITIS (PAM) soil-water ameboflagellate, swimming in warm bodies of water

GIARDIASIS contaminated water supplies, beavers, malabsorption syndrome

TRICHOMONIASIS sexually transmitted disease (STD), metronidazole

AFRICAN TRYPANOSOMIASIS sleeping sickness, tsetse flies, variation of surface antigens

AMERICAN TRYPANOSOMIASIS Chagas' disease, kissing bugs, Romaña's sign

LEISHMANIASIS visceral (kala-azar), cutaneous (Oriental sore), mucocutaneous, sandflies

PLASMODIASIS malaria, female *Anopheles* mosquito, preerythrocytic (liver) stage, thick and thin smear, chloroquine and primaquine phosphate

TOXOPLASMOSIS fetal teratogen, cat family

PNEUMOCYSTOSIS fatal pneumonia in the immunosuppressed

VISCERAL LARVA MIGRANS (VLM) ascarids (roundworms) of dogs and cats, pica

HOOKWORM barefootedness, anemia

CUTANEOUS LARVA MIGRANS ground itch, larvae of dog and cat hookworms

STRONGYLOIDIASIS rhabditiform larvae that transform into filariform (infective, parasitic) larvae or into a free-living generation of adults

ENTEROBIASIS pinworm, seatworm, anal pruritus, transparent tape swab method

TRICHINOSIS (TRICHINELLIASIS) muscle worm, periorbital edema, pork

FILARIASIS nocturnal periodicity, elephantiasis

SCHISTOSOMIASIS blood flukes, camouflage with host proteins, snails; schistosome dermatitis—swimmers' itch

TAENIASIS pork and beef tapeworms, bladder worm infection (cysticercosis)

ARTHROPODA vectors, intermediate hosts, venenators, ectoparasites

QUESTIONS FOR STUDY

1. Define the term *parasite* and explain the difference in the usage of the term when applied to bacteria and viruses as opposed to infectious agents of the animal kingdom.
2. Discuss factors that affect the distribution and incidence of parasitic agents and parasitic disease.
3. How does the laboratory diagnosis of parasitic disease differ from that of bacterial and viral diseases?
4. Serological testing for parasitic infection is not as rewarding as it is for bacterial and viral diseases. Explain.
5. Discuss some of the problems associated with the control of parasitic disease agents.
6. Diffferentiate between parasitic infection and parasitic disease.
7. Define *definitive host, intermediate host, ectoparasite, endoparasite.*

8. What word ending (suffix) of the name of a disease almost always distinguishes a parasitic disease from bacterial, viral, or fungal diseases?
9. Give a brief differentiating description for the four divisions of the animal kingdom that contain parasitic disease agents.
10. Give the name of the causal agent of the following protozoal diseases, a description of the disease, the mode of transmission, and the treatment:

    amebiasis
    giardiasis
    primary amebic meningoencephalitis
    vaginal trichomoniasis
    plasmodiasis
    trypanosomiasis
    leishmaniasis
    balantidiasis
    toxoplasmosis

11. Which protozoan is the causal agent of a prevalent sexually transmitted disease?
12. Which protozoan is a noted fetal teratogen?
13. Which protozoan is noted for causing oftentimes fatal pneumonia in immunocompromised individuals (e.g., transplant recipients, AIDS patients)?
14. Associate the following vertebrates or invertebrates with the parasitic disease agent for which they serve as hosts or as reservoirs: cat, swine, beaver, female *Anopheles* mosquito, tsetse fly, kissing bug.
15. Give the name of the causal agent of the following roundworm (nematode) diseases, a description of the disease, the mode of transmission, and the treatment: ascariasis, hookworm infection, strongyloidiasis, enterobiasis, trichinelliasis.
16. With which parasitic diseases are the following associated: pica, going barefoot, ground itch, seatworm infection, transparent tape swab, anal pruritus, muscle worm infection, cysticercosis or bladder worm infection, northern lake trout, periorbital edema?
17. Cite factors that have contributed to the decline in incidence of trichinelliasis.
18. What are flukes?
19. What is the layman's term for schistosome dermatitis? Why is the infection with schistosomes limited to the skin?
20. Wherein lies the real danger of a pork tapeworm infection?
21. Cite four ways in which arthropods are important medically.

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IX. SELECTED TOPICS

37. ORAL MICROBIOLOGY

OBJECTIVES

To list the sites or microcosms of the oral cavity that have the greatest concentrations of microorganisms

To characterize in general terms and give the names of the microorganisms that colonize these oral sites

To identify which of the oral bacteria are prime suspects in causing caries and periodontal disease in man

To describe adherence of *Streptococcus mutans* to enamel surfaces and its role in human dental caries

To discuss the role of the acquired pellicle, extracellular polysaccharides, intracellular storage polysaccharides, and secretory IgA in plaque formation and caries

To describe efforts to control human cariogenic microorganisms or to modify their effect on tooth enamel

To characterize in general terms the microorganisms that may be listed as periodontopathic and to discuss the host's response to them

To discuss bacterial activities or products that contribute to inflammation of gingivae and periodontium

Development of the Oral Microflora
Microflora of the Gingival Crevice
Microflora of the Tongue
Microbial Composition of Dental Plaque
Factors Affecting Development of the Oral Microflora
Microbial Interactions
Adherence
Bacterial and Epithelial Surfaces
Adherence of *Streptococcus mutans* to Enamel Surfaces
Adherence of Oral Streptococci to Oral Epithelial Surfaces
Salivary Glycoproteins and the Acquired Pellicle
Secretory IgA and Interference with Colonization
Characteristics of Important Oral Microorganisms
The Streptococci
The Veillonellae
The Lactobacilli
The Actinomycetes
The Bacteroides
The Fusobacteria
The Spirochetes
The Diptheroids
Dental Caries
Factors Important in Caries Formation
Pellicle Formation
Plaque Formation
The Nature of Plaque
The Role of *Streptococcus mutans*
Microbial Manipulation of Dietary Sucrose
Host Factors in Caries or Plaque Control
Treatment and Control
Sucrose Substitutes
Fluoridation
Antimicrobial Agents
Occlusal Sealants
Periodontal Disease
Factors Important in Periodontal Disease
Role of Microbial Products
Role of the Host
Acute Necrotizing Ulcerative Gingivitis

Quantitative and qualitative studies on the microorganisms indigenous to the oral cavity have in most instances been frustrating and difficult. Some areas of the mouth are not easily accessible for the collection of plaque, which is the soft adhesive aggregation of bacteria and their products on the tooth surface. Sampling techniques have, therefore, not been uniform, and results have been misleading. In many early studies, supragingival and subgingival plaque specimens were often pooled, as were plaques from different teeth or from different sites on the same tooth, the assumption being that all dental plaques were the same. We now know that this is not the case. Although there may be considerable overlap in the microbial flora in these specimens there are also microbes that are unique to specific sites. Sampling techniques have therefore not been uniform, and results have been misleading. Furthermore, almost 50 percent of the cultivable microorganisms in the oral cavity are anaerobes. Only recently have convenient techniques that permit the isolation of the very oxygen-sensitive anaerobes been devised. As techniques for the isolation and identification of microorganisms improve, more microbial types will undoubtedly be recovered and identified. Estimates of the number of microbial species colonizing the adult human oral cavity approach 300. Regardless of the difficulties, a substantial amount of information has accumulated about the ecology of the oral cavity that helps us to understand some of the relationships between microorganism and host in health and disease.

The primary purpose of this chapter is to discuss in greater detail the causal agents of diseases that are manifested only in the oral cavity: dental caries and periodontal disease. Caries (tooth decay) is a disease of the dental hard tissue, while periodontal disease is a disorder of the tissue surrounding the teeth (Fig. 37-1).

Fig. 37-1. Sagittal section of a tooth and its attachments.

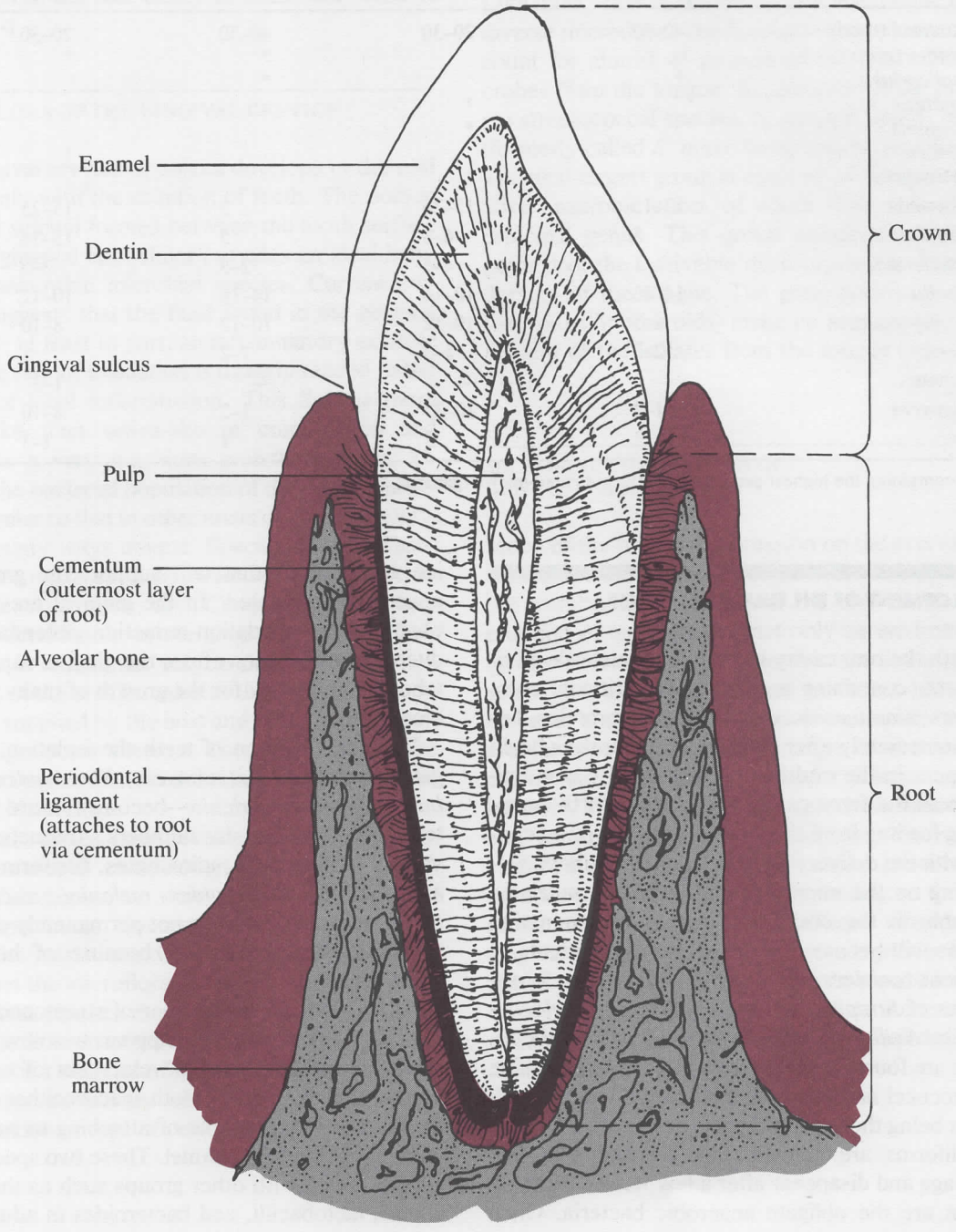


Table 37-1. Percentages of Cultivable Microorganisms Isolated from Various Areas of the Oral Cavity

Organism or group	Tongue	Plaque	Saliva	Gingival crevice
Streptococci (total)	40–50	20–30	40–50	20–30
S. mitior (mitis)			*	*
S. salivarius	*		*	
S. mutans		*		
S. sanguis		*		
S. milleri				*
Enterococci		*		
Diphtheroids	12–15	15–20	5	15–25
Bacteroides	5	6–8	5	15–18
Fusobacterium	1	4–5	2–3	3–5
Veillonella	14–16	10–14	14–16	10–12
Peptostreptococcus	4–5	10–12	10–12	8–10
Neisseria	2–3	1–3	1–2	1
Spirochetes	—	—	—	1–3
Actinomyces	1–2	30–35	1–2	8–10
Vibrio	2	2	1	1–3

*Area containing the highest percentage of the indicated microorganism.

DEVELOPMENT OF THE ORAL MICROFLORA

At birth the oral cavity is essentially a sterile environment, containing a variety of nutritional components: amino acids, carbohydrates, proteins, lipids. Immediately after birth, microorganisms begin to appear in the oral cavity, acquired by contamination as the fetus passes through the birth canal, during feeding from the mother's breast, or by contact with the delivery team or family members. Depending on the microorganisms and the nutrients available in the oral cavity, these contaminating species will become either permanent residents or transient travelers. Within 3 or 4 days after birth, species of *Streptococcus, Neisseria, Staphylococcus*, and *Veillonella* as well as lactobacilli and coliforms are found in the mouths of most infants. The streptococci are in greatest numbers, with *S. salivarius* being the predominant species.

Coliforms are characteristic transients at this early age and disappear after a few weeks. Notably absent are the obligate anaerobic bacteria. Once teeth have erupted, an environment (gingival crevice and plaque) that will support the growth of anaerobes is created. In the gingival crevice, for example, the oxidation-reduction potential is reduced, and secretions from the gingiva supply metabolites necessary for the growth of many anaerobic species.

With the eruption of teeth the isolation of obligate anaerobes—*Actinomyces, Bacteroides, Fusobacterium, Leptotrichia*—becomes more consistent. An increase in numbers, frequency, and variety is seen with spirochetes, fusiform bacilli, and vibrios. *Bacteroides melaninogenicus* and some oral spirochetes do not permanently colonize until puberty, presumably because of hormonal factors.

Although the total number of streptococci does not change when the teeth appear, two new species are associated with the development of teeth: *S. mutans* and *S. sanguis*. Both species either directly or indirectly are capable of attaching to hard surfaces such as dental enamel. These two species disappear, along with other groups such as the spirochetes, lactobacilli, and bacteroides in adults who are edentulous (without teeth). Some of the anaer-

obic forms reappear along with *S. sanguis* and *S. mutans* if dentures are worn. The groups of microorganisms in the oral cavity of adults are listed in Table 37-1.

MICROFLORA OF THE GINGIVAL CREVICE

The gingival crevice or sulcus develops in the oral cavity only with the eruption of teeth. The pocket (gingival sulcus) formed between the tooth surface and the gingival epithelium provides an ideal habitat for anaerobic microbial species. Current evidence suggests that the fluid found in the gingival sulcus is, at least in part, an inflammatory exudate since the rate of exudation is directly related to the degree of local inflammation. This fluid is more serum-like than saliva-like in composition and serves as a very nutritious growth medium. Although the bacterial population of the gingival crevice is similar to that in other areas of the oral cavity, it is in many ways unique. Species such as *Fusobacterium nucleatum, B. melaninogenicus, Treponema macrodentium*, and *Campylobacter sputorum* are found only in the gingival crevice. Their presence is related not only to the anaerobic environment of the area but also to the nutritional components supplied by the host and other microorganisms indigenous to the area. These relationships are discussed under Factors Affecting Development of the Oral Microflora.

Several studies have revealed a relatively consistent microbial composition of the sulcal flora. These data, however were derived from pooled plaque specimens. Consequently, tooth-to-tooth or site-to-site variations were obscured. Today we know that the microflora differ in supragingival and subgingival plaque, in plaque from different teeth and from different sites on a given tooth. The proportions of a number of microorganisms have also been observed to vary with the degree of gingival inflammation or type of periodontitis.

MICROFLORA OF THE TONGUE

Like most areas of the oral cavity, the tongue has a diverse microflora. The facultative streptococci account for almost 45 percent of the cultivable microbes from the tongue. *S. salivarius* is the principal streptococcal species, *S. sanguis* and *S. mitior* (formerly called *S. mitis*) being minor constituents. The next largest group is made up of the gram-negative anaerobic cocci, of which *Veillonella* is the principal genus. This group comprises about 16 percent of the cultivable microorganisms from the dorsum of the tongue. The gram-positive facultative rods (diphtheroids) make up approximately 13 percent of the isolates from the tongue (see Table 37-1).

MICROBIAL COMPOSITION OF DENTAL PLAQUE

Much of the previous discussion on the oral microbiota has dealt with microorganisms that appear on the membranous surfaces of the oral cavity. The eruption of teeth creates not only an environment for the development of anaerobic species (gingival sulcus) but also a hard enamel surface, which may serve as a site of attachment for a variety of microbial species. Once teeth begin to appear, the enamel surface becomes a site for colonization by bacteria. The soft, adhesive aggregation of bacteria and extracellular polysaccharides on the enamel surface is referred to as *dental plaque*. It contains a number of microbial species, depending on the area of the tooth surface sampled. Plaque close to the gingival margin, for example, has a higher percentage of anaerobic species than does plaque located on a buccal surface. Table 37-2 lists some of the major groups and species of bacteria isolated from dental plaque.

Table 37-2. Major Groups of Microorganisms Isolated from Plaque

Group	Mean percentage*
GRAM-POSITIVE FACULTATIVE COCCI	28.2
Streptococcus mitior (mitis)	
S. milleri	
S. salivarius	
S. sanguis	
S. mutans	
GRAM-POSITIVE ANAEROBIC COCCI	12.6
Peptococcus	
Peptostreptococcus	
GRAM-NEGATIVE ANAEROBIC COCCI	6.0
Veillonella alcalescens	
V. parvula	
GRAM-NEGATIVE FACULTATIVE COCCI	0.4
Neisseria flava	
N. sicca	
N. flavescens	
N. catarrhalis	
GRAM-POSITIVE FACULTATIVE RODS	23.8
Actinomyces naeslundii	
A. viscosus	
A. israelii	
A. odontolyticus	
Rothia dentocariosa	
Bacterionema matruchotii	
GRAM-NEGATIVE FACULTATIVE RODS	<1
Hemophilus species	
GRAM-POSITIVE ANAEROBIC RODS	18.4
Leptotrichia buccalis	
Eubacterium saburreum	
GRAM-NEGATIVE ANAEROBIC RODS	10.4
Bacteroides melaninogenicus	
B. intermedius	
B. gingivalis	
Fusobacterium nucleatum	
Campylobacter sputorum	

*Data on pooled samples combined from several sites. Source: Adapted from S. S. Socransky and A. D. Manganiella, The oral microbiota of man from birth to senility. *J. Periodontol.* 42:485, 1971.

FACTORS AFFECTING DEVELOPMENT OF THE ORAL MICROFLORA

Microbial Interactions

It is difficult to assess the effect that one microorganism or a group of microorganisms has on other microorganisms in any ecological niche. In humans the microflora of the oral cavity is made up of hundreds of species of microorganisms. Each species controls, to some extent, and is controlled by the environment in which it is harbored. Although one cannot extrapolate in vitro results to the in vivo situation, some interrelationships between oral microorganisms have been noted. These relationships may play some part in the establishment of certain microbial species.

1. *Treponema macrodentium* is an oral spirochete that can grow on laboratory media when cultivated in the presence of other gingival microorganisms. The most important of the supporting organisms are a diphtheroid and a fusobacterium. When these two are present in the medium, their growth products are capable of lowering the oxidation-reduction potential of the medium to a level (−185 millivolts) that supports the growth of the spirochete. Neither supporting organism alone can reduce the medium to the necessary level for the growth of the spirochete. In addition, the isobutyrate produced by the supporting organisms provides a growth factor required by *T. macrodentium*.
2. *Campylobacter sputorum* is also an inhabitant of the gingival crevice. Hydrogen is an important energy source because this organism does not ferment amino acids or carbohydrates. In vitro results suggest that formate can substitute for this hydrogen requirement. Several gingival microorganisms including *Bacteroides oralis, Fusobacterium nucleatum*, and *Bacteroides melaninogenicus* can produce formate.
3. Some oral *Veillonella* species cannot ferment carbohydrates but do metabolize a common product of carbohydrate fermentation, lactate. Lactate is produced by many bacterial species such as the streptococci. Evidence that this fer-

mentation product is required is the inability of *Veillonella* species to establish themselves in germ-free animals.

4. *S. mutans* requires para-aminobenzoic acid (PABA) for growth; *S. sanguis* does not. In a culture medium free of PABA, *S. sanguis* supports the growth of *S. mutans*. Thus the PABA produced by *S. sanguis* or by other microbial species in the oral cavity may control the level of growth of *S. mutans*.
5. The black-pigmented *Bacteroides*, important periodontopathogens, are stimulated by growth factors produced by *Veillonella*, anaerobic streptococci, and gram-positive facultative bacilli. These growth factors include succinate and naphthoquinone, a compound related to vitamin K.
6. Oral colonization is also affected by negative or antagonistic interactions between microbes. These may be in the form of bacteriocins, the antimicrobial proteins produced by some bacteria. The black-pigmented *Bacteroides* are inhibited by bacteriocin-like molecules produced by *S. sanguis* and *S. mitior* and in turn produce bacteriocins that inhibit *S. mitior, S. salvarius*, and certain other *Bacteroides, Capnocytophaga*, and *Actinomyces* species. A bacteriocin of *S. sanguis* is inhibitory to the causal agent of localized juvenile periodontosis, *Actinobacillus actinomycetemcomitans*.
7. Many groups of bacteria—streptococci, diphtheroids, fusobacteria—are capable of producing and storing intracellular polysaccharides (amylopectin, a glycogen-like compound) as well as extracellular polysaccharides such as glucans (dextran and mutan) and fructans (levan). The intracellular carbohydrate reserves help maintain the indigenous population of microorganisms, particularly when the dietary carbohydrate intake is restricted. The extracellular polysaccharides dextran and levan are readily hydrolyzed and fermented by many oral microbial species. Moreover, metabolism of these intracellular and extracellular reserves in plaque by cariogenic (cavity-producing) streptococci such as *S. mutans* aids in maintaining the pH of plaque at decalcifying levels and contributes to development of caries.

Adherence

From current studies it now appears that many factors, both chemical and physical, determine the kind and number of microorganisms that inhabit the ecological niches of the oral cavity. Nutrition, of course, plays an important part in determining which microorganisms can survive in any area of the oral cavity. But why do organisms of the same genus and with similar metabolic activities differ in the type of oral surface they colonize? Why does *S. mitior*, for instance, preferentially colonize the smooth buccal surface while *S. salivarius* has an apparent affinity for the dorsum of the tongue? For colonization of the oral surface to occur, bacteria must first become attached to the surface and then must be capable of growth. Because of epithelial desquamation and salivary cleansing, large biomasses are not ordinarily seen on oral surfaces, and many bacteria become desorbed from them. It stands to reason that some mechanism, or mechanisms other than nutrition, must exist that will allow an organism to attach to and subsequently colonize oral surfaces. The property of adherence now seems to be a major determinant for retention of microorganisms in the oral cavity as well as other anatomical sites. Adherence may be associated with the composition of the bacterial surface or the epithelial surface to which the microorganism attaches. The composition of the saliva in which microorganisms are constantly bathed may also play an important role in adherence. The synthesis of the extracellular polysaccharides dextran and mutan are essential for the adherence of *S. mutans* to smooth enamel surfaces.

BACTERIAL AND EPITHELIAL SURFACES. According to several studies, certain surface components on bacterial cells enable bacteria to adhere to epithelial surfaces. Electron micrographs show that *S. salivarius, S. pyogenes*, and *S. mitior* all have a "fuzzy" fibrillar coat. In *S. pyogenes* this coat is associated with the virulence of the organism and is called the *M protein*. Presumably it accounts in

part for the ability of *S. pyogenes* to adhere to epithelial surfaces.

The ability of *S. pyogenes* and *S. salivarius* to adhere to epithelial cells of the oral mucosa is impaired or totally lost if the cells of these species have been pretreated with trypsin, wheat germ lipase, polysorbate (Tween) 80, sodium lauryl sulfate, antisera, or heat. Thus the bacterial surface determinant appears to be composed of protein and lipid and to be heat labile.

The specificity between bacterial cell and oral surface must also be governed by the character of the epithelial cell. One study compared virulent *S. pyogenes* and an enteropathogenic strain of *Escherichia coli*. The enteropathogenic *E. coli* contains a surface antigen called *K88* that functions in adherence. Electron micrographs show it to be composed of long filaments that differ greatly from the fuzzy components of *S. pyogenes*. Virulent *S. pyogenes* adhered in higher proportions than *E. coli* to mucosal surfaces of rat tongues. On the mucosal surfaces of the urinary bladder, however, the situation was reversed: *E. coli* demonstrated a greater capacity for adherence to bladder cells than did *S. pyogenes*. Thus adherence depends both on the type of epithelial surface and on the surface chemistry of the microbial species.

ADHERENCE OF *STREPTOCOCCUS MUTANS* TO ENAMEL SURFACES. *S. mutans*, when compared with other streptococcal species, has essentially no adsorptive qualities for epithelial cells or for enamel. Yet this organism is found in relatively high numbers in dental plaque and in carious lesions. Its adsorptive power is related to its ability to produce gelatinous masses of polysaccharide when the diet is high in sucrose. The colonization and aggregation of this cell type is directly related to the synthesis of insoluble extracellular polysaccharides, specifically mutan. Thus a mechanism is available for the attachment of *S. mutans* to the enamel surface, a mechanism considered by many to be the most important virulence factor of *S. mutans*.

ADHERENCE OF ORAL STREPTOCOCCI TO ORAL EPITHELIAL SURFACES. The distribution of oral streptococci on oral epithelial surfaces apparently is correlated with the ability of the species to adhere to such surfaces.

1. *S. sanguis* and *S. salivarius* are present in large numbers on oral epithelial surfaces. Both have shown a greater capacity than other streptococcal species to adhere in vitro to epithelial cells from cheek scrapings of humans, hamsters, and germ-free rats.
2. *S. mutans* is not found on epithelial surfaces in vivo and in vitro adheres poorly or not at all to human or animal epithelial cells.
3. *S. salivarius* is isolated in greater numbers from the dorsum of the tongue than is *S. sanguis*, even though both apparently have the same growth requirements. In vitro *S. salivarius* shows a greater affinity for epithelial cells of the tongue than does *S. sanguis*. The reverse is true on the surface of the tooth: *S. salivarius* adheres poorly to enamel, while *S. sanguis* adheres readily. *S. sanguis* is often described as the predominant streptococcal species in noncarious plaque.

SALIVARY GLYCOPROTEINS AND THE ACQUIRED PELLICLE. It has been known for years that saliva can cause the aggregation of bacteria. The salivary factor that causes the aggregation was found to be a glycoprotein. Glycoproteins are high molecular weight compounds consisting of a conjugated protein in which the prosthetic groups are carbohydrates. Although the carbohydrates make up less than 10 percent of the molecular weight of the glycoprotein, they greatly affect its properties and functions. The glycoprotein fraction of saliva causes the aggregation of oral streptococci, and it is selectively adsorbed to hydroxyapatite in tooth enamel. Adsorbed salivary glycoproteins constitute the acquired pellicle on the tooth surface. Many researchers believe that the interbacterial matrix of dental plaque is a composite of salivary glycoproteins and bacterial polysaccharides.

The importance of the acquired pellicle for bacterial adherence to the tooth surface is well documented. The in vitro attachment of *S. sanguis*, *S. salivarius*, and *S. mutans* to uncoated and saliva-

coated enamel was compared. Very few bacteria adhered to the surface of uncoated enamel. The presence of an acquired pellicle on the enamel surface, however, enhanced the attachment of all species, but not equally. *S. sanguis* attached better than *S. mutans*, and both surpassed *S. salivarius*. This result indicated that the degree of adherence was species specific and that the bacterial surface showed varying affinities for the salivary glycoproteins.

In another study both *S. sanguis* and *S. mitior* were aggregated by whole saliva. The salivary aggregation factor, or at least part of it, affected both species. *S. sanguis* removed all of the factor from saliva that aggregated *S. sanguis* but removed only 36 percent of the factor needed for aggregation of *S. mitior*. In another study the abilities of *S. sanguis* and *S. salivarius* to adhere to enamel powder and saliva-coated enamel powder were compared. Both species adhered to enamel powder. The presence of salivary glycoproteins, however, interfered with the adsorption of *S. salivarius* and increased the adsorption of *S. sanguis*. Thus the effect of whole saliva on adherence of bacterial species depends on the nature of the oral glycoprotein-coated surface. Salivary glycoproteins have also been shown to decrease the adherence of *S. salivarius* to the acquired pellicle. These same salivary glycoproteins, however, did not affect the attachment of *S. salivarius* to human cheek cells. Although some of these results indicate that agglutination of bacteria by salivary glycoproteins hampers their ability to attach to oral surfaces, other factors in the saliva may also be involved.

SECRETORY IgA AND INTERFERENCE WITH COLONIZATION. A local immune system does exist in the oral cavity. It is composed of a special immunoglobulin called *secretory IgA* (*sIgA*). Secretory IgA is found in colostrum, tears, and saliva. It is the major immunoglobulin in whole saliva and thus represents the predominant class of antibody bathing mucosal surfaces. The protective effect of sIgA has been clearly demonstrated with certain viruses, particularly poliovirus, rhinovirus, and influenza virus. The antibacterial effect of sIgA is less well documented. In fact, sIgA is not considered bactericidal. It does not enhance phagocytosis or mediate complement-dependent bacterial lysis. Many microbiologists believe that any antibacterial effect of sIgA is due to its ability to interfere with bacterial colonization by preventing attachment to epithelial surfaces. There is evidence supporting this hypothesis. In one study coproantibodies (antibodies in the intestines) containing sIgA were obtained from the intestinal tract of mice that had been orally vaccinated with cholera vaccine. The coproantibodies reduced the number of cholera vibrios that could be adsorbed to the intestinal mucosa of unvaccinated and cholera-susceptible mice. In another study sIgA obtained from parotid saliva inhibited the attachment of only those oral bacterial species that the immunoglobulin could agglutinate. sIgA had no effect on adherence of species that it could not agglutinate—in particular, species not indigenous to the oral cavity. Antibodies are apparently synthesized locally and do not interfere with organisms indigenous to other areas of the body. There is some evidence that inhibition of adherence extends to the cariogenic species *S. mutans*. *S. mutans* produces an extracellular glucan that resembles dextran. The glucan is synthesized through the action of the enzyme glucosyltransferase on sucrose. This glucan is responsible for the aggregation of *S. mutans* cells. Glucosyltransferase bound to *S. mutans* cells facilitates the attachment of *S. mutans* to glass, wires, and the tooth surface. Antibody directed to whole *S. mutans* cells or to glucosyltransferase prevents attachment to glass or wire surfaces, reduces caries incidence in experimental animals, and reduces colonization of tooth surfaces in human studies.

CHARACTERISTICS OF IMPORTANT ORAL MICROORGANISMS

THE STREPTOCOCCI

The streptococci are the predominant group of microorganisms in the oral cavity. The viridans group

Table 37-3. Major Properties Used to Separate the Viridans Strains of Streptococci

Property	Strain					
	S. pneumoniae	*S. salivarius*	*S. mitior*	*S. milleri*	*S. sanguis*	*S. mutans*
Sensitivity to						
Optochin	S	R	R	R	R	R
Nitrofurazone (10 μg)	S	R	SR	R	SR	R
Growth						
On 10% bile agar	−	+	−	±	±	+
In 4% NaCl broth	−	−	−	−	−	+
Acid from						
Mannitol	−	−	−	−	−	+
Sorbitol	−	−	−	−	−	+
Salicin	−	+	−	+	+	+
Ability to synthesize glucans and fructans from sucrose	−	+	−	−	+	+
Hydrolysis of						
Esculin	−	+	−	+	±	±
Arginine	−	−	−	+	+	−
Cell wall contains						
Rhamnose	−	+	−	+	+	−
Ribitol	−	−	+	−	−	−

+ = usually positive; − = usually negative; ± = over 60% of isolates are positive; S = sensitive; R = resistant; SR = over 60% of isolates are sensitive.
Source: Adapted from G. Colman and R. E. O. Williams, Taxonomy of Some Human Viridans Streptococci. In L. W. Wannamaker and J. M. Matsen (Eds.), *Streptococci and Streptococcal Diseases*. New York: Academic, 1972.

occurs with the greatest frequency and includes the following species: *S. mitior, S. salivarius, S. milleri, S. mutans, S. sanguis*, and *S. pneumoniae*. *S. pneumoniae* was discussed at length in Chapter 19. Other important oral streptococci include the enterococci and members of the genera *Peptococcus* and *Peptostreptococcus*. Differentiation of the viridans streptococci is described in Table 37-3.

S. salivarius constitutes a high percentage of microorganisms found on the tongue, in the saliva, and on the buccal mucosa. Large mucoid colonies are produced on media containing sucrose as the result of synthesis of the extracellular polysaccharide levan via metabolism of sucrose. Levan produced by other microorganisms is a component of plaque and is readily hydrolyzed by a variety of plaque microorganisms. Levan thus serves as a source of fermentable carbohydrate during periods of low dietary sucrose intake. *S. salivarius* is of no importance in caries formation in humans.

S. sanguis can be found in large numbers in dental plaque, but it has not been shown to be involved in human caries formation. Like *S. salivarius*, it produces large amounts of extracellular polysaccharide, primarily dextran, on media containing sucrose.

S. milleri is a recently accepted species that was once originally classified with *S. mitis*. It has been

found in highest numbers in the gingival crevice.

S. mitior (formerly *S. mitis*) is present in most areas of the oral cavity. In germ-free rats it can cause fissure caries. It plays no role in human caries.

S. mutans is found primarily on smooth enamel surfaces and in pits and fissures. It is capable of producing extracellular glucans and fructans from dietary sucrose and is considered by most to be the primary human cariogenic microorganism. While other streptococci and oral microorganisms produce extracellular polysaccharides, only *S. mutans* produces the insoluble glucan known as mutan. It also expresses glucan receptors on its surface that facilitate its aggregation and adherence to the tooth surface. This ability to adhere to the tooth surface is considered by many to be the principal virulence factor contributing to development of smooth surface caries. *S. mutans* can be differentiated from other members of the viridans streptococci by its ability to ferment sorbitol and mannitol.

It has been difficult to separate *S. mutans* into distinct strains or subspecies since many appear to be heterogeneous in response to most physiological tests. Currently, three methods are used to distinguish between the subspecies: (1) serotyping of cell wall carbohydrate antigens, (2) DNA homology, and (3) percent guanine and cytosine in DNA. The serological approach is the most convenient. This method, first employed by Brathall, has been used to describe seven serological types.

Endocarditis caused by *S. mutans* occurs infrequently and is seen primarily in persons with heart disease. In this regard it is important to differentiate *S. mutans* as well as other alpha-hemolytic streptococci from the enterococci because of the difference in therapy—group D enterococci are usually resistant to penicillin. The role of *S. mutans* in dental caries is discussed later in the chapter.

The enterococci belong to Lancefield group D. Approximately 60 to 75 percent of the human population carry enterococci in the oral cavity. The species most often isolated is *S. faecalis*. They have been implicated in a number of diseases, including meningitis, endocarditis, mastoiditis, and peritonitis. Enterococci have been found in infected root canals and also participate in Ludwig's angina, a serious infection of mixed etiology involving the floor of the mouth. As mentioned above, it is important to distinguish enterococci from the viridans streptococci because of the former's resistance to penicillin. Penicillin is effective against enterococci when it is administered in conjunction with streptomycin.

Peptococcus and *Peptostreptococcus* are two genera of anaerobic gram-positive streptococci found in the oral cavity. Species from both genera are biochemically unreactive. Speciation requires identification of metabolic products by gas-liquid chromatography. They are not primary causal agents in periodontal disease or caries. They can be aspirated into the lung and can initiate pleuropulmonary infections in alcoholics and persons under anesthesia.

THE VEILLONELLAE

The veillonellae are gram-negative anaerobic cocci that appear as diplococci and in small chains or masses. They are the second most numerous group of bacteria isolated from the oral cavity. They are readily found in dental plaque, but their role in caries and periodontal disease is not known. In animal model systems, glucans produced by other microorganisms such as *S. mutans* appear to be responsible for the attachment of veillonellae to plaque. Lactate produced by streptococcal species can be metabolized by the veillonellae. It has been hypothesized that these characteristics actually reduce the cariogenicity of *S. mutans* by preventing the lactic acid demineralization of enamel.

THE LACTOBACILLI

The lactobacilli are gram-positive, nonmotile rods found in the intestinal tract, vagina, and oral cavity of humans. They are microaerophilic or anaerobic, with complex nutritional requirements. The number of lactobacilli in the oral cavity is low, seldom exceeding 2×10^5 viable cells per milliliter of sa-

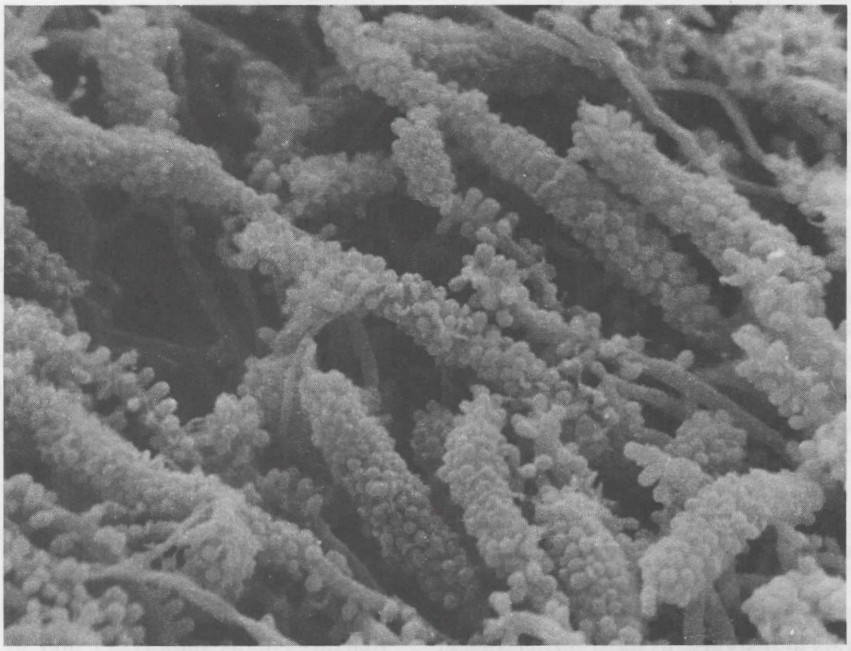


Fig. 37-2. Scanning electron micrograph demonstrating "corncob" formations in plaque. Filamentous microorganisms are covered by coccoid bacterial forms. (From S. J. Jones, *Arch. Oral Biol.* 17:613, 1972.)

liva. This low number may be due to their inability to attach to oral surfaces. The low pH of caries-associated plaque favors the accumulation of lactobacilli. For many years lactobacilli were believed to be the primary causal agent of caries because they are acidogenic and can tolerate acid pH as well as grow at pH's that initiate demineralization of enamel. However, they comprise at most only about 0.5 percent of the plaque microflora and are much less acidogenic than *S. mutans*. They are not considered to be initiators of human caries, although they may contribute to the progression of the carious lesion. In some experiments germ-free rats were intentionally colonized with lactobacilli species and caries resulted. While these experiments may demonstrate the potential of lactobacilli to cause caries it is not believed that lactobacilli are efficient cariogenic microorganisms in hosts bearing a normal oral microflora.

THE ACTINOMYCETES

The actinomycetes are gram-positive filamentous branched bacteria of which *Actinomyces israelii* is the most important pathogenic species (see Chapter 31). Other *Actinomyces* species such as *A. odontolyticus*, *A. viscosus*, and *A. naeslundii* have at one time or another been implicated in root surface caries or periodontal disease. Their role in these diseases is not understood. Both *A. naeslundii* and *A. viscosus* can produce caries in germ-free rats. For these species to become part of the plaque microbiota, certain bacterial and salivary factors must be present. Salivary glycoproteins are necessary for attachment to the tooth surface. Further, plaque examined by transmission electron microscopy has revealed cocci attached to the outer surface of filamentous bacteria by a capsule-like material of apparent coccal origin (Fig. 37-2). Both *A.*

naeslundii and *A. viscosus* are capable of agglutinating *S. sanguis* and *S. mitior*, normal residents of plaque. In animal models both of these *Actinomyces* species can cause periodontal disease.

THE BACTEROIDES

The genus *Bacteroides* is made up of gram-negative, obligate anaerobic rods or coccobacilli. In the oral cavity they reside in the gingival sulci and in subgingival plaque. Three species most frequently isolated from the oral cavity are *B. melaninogenicus, B. intermedius*, and *B. gingivalis*. They are characterized by their ability to produce a black pigment when cultivated on blood agar, and most strains require vitamin K or structurally related compounds for growth.

B. melaninogenicus is most often isolated from healthy sulci, whereas the other two species are strongly associated with periodontitis, acute necrotizing ulcerative gingivitis (ANUG), and other inflammatory conditions of the gingivae. Destruction of periodontal tissue is initiated by the production of toxic wastes and lytic enzymes: NH_3, H_2S, indole, collagenase, fibrinolysin, and immunoglobulin proteases. The bacteroides also inhibit neutrophil chemotaxis and phagocytosis and resist complement-mediated killing.

THE FUSOBACTERIA

The fusobacteria are gram-negative anaerobic nonsporeforming rods. Although *fuso* means "spindle shaped" (elongated cells with tapered ends), only some of the species assume that shape in culture or in clinical specimens. The oral fusobacteria are classified into one species, *Fusobacterium nucleatum*. They are frequently found in the gingival crevice and dental plaque. Their numbers as well as those of the spirochetes rise dramatically in inflammatory periodontal disease. The increase may be due to the inflammatory condition itself and does not necessarily mean that they are the cause of the disease.

THE SPIROCHETES

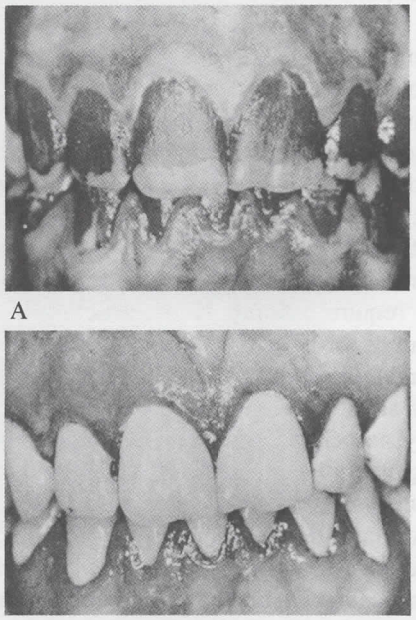
The spirochetes are restricted to the gingival crevice because of stringent anaerobic requirements. Two genera are recognized, *Treponema* and *Borrelia*, but, because of difficulty in culturing, most researchers classify spirochetes as small, intermediate, or large. Five species of small and intermediate spirochetes have been cultivated and described: *T. denticola, T. macrodentium, T. scoliodontum, T. oralis*, and *T. vincentii*. *T. vincentii* was first described by Vincent in cases of ANUG. Oral treponemes have a narrow oxidation-reduction potential range (-180 to -220 millivolts) and cannot survive partial pressures of oxygen above 0.3 percent. It is the need for a low oxidation-reduction potential that restricts them to the gingival crevice. Except for *T. macrodentium*, they do not ferment carbohydrates but can ferment amino acids. As noted with the fusobacteria, the spirochetes greatly increase their numbers in inflammatory periodontal disease. It has been suggested that this increase, rather than implicating them as primary agents of infection, results from a lowering of the oxidation-reduction potential in the infected area.

THE DIPHTHEROIDS

The diphtheroids are an ill-defined group composed of species of the genera *Corynebacterium* and *Propionibacterium*. Anaerobic species are included in the latter genus. Approximately 20 percent of the cultivable microorganisms in dental plaque are diphtheroids. Their relationship to periodontal disease has not been established. They do, however, produce chondroitin sulfatase, an enzyme capable of hydrolyzing chondroitin sulfate; this polysaccharide is a component of connective tissue surrounding and adhering to teeth.

Fig. 37-3. Rampant caries. (Courtesy Program in Dental Hygiene, Marquette University School of Dentistry.)

Fig. 37-4. Plaque accumulation on teeth. A. Heavy accumulations of plaque evidenced by staining with basic fuchsin. B. Appearance of teeth after basic fuchsin application and removal of plaque. (From I. Glickman, *Clinical Periodontology* [3rd ed.]. Philadelphia: Saunders, 1964.)



A

B

DENTAL CARIES

Caries (tooth decay) is a bacterial disease characterized by demineralization of the tooth surface (Fig. 37-3). Most of the evidence to date indicates that diet and specific microbial species are correlated with the high incidence of this universal disease. Because of the difficulty in establishing infections in human volunteers, no clear-cut relationship between the causal agent or agents and the disease has been demonstrated in humans. However, certain physical, chemical, and microbiological factors appear to be important in caries formation in humans as well as in animals.

FACTORS IMPORTANT IN CARIES FORMATION

Pellicle Formation

A few hours after cleansing the teeth by flossing or toothbrushing, an acellular, structureless material begins to form on the tooth surface. This material is called the *acquired pellicle*. The pellicle is organic, consisting primarily of soluble salivary glycoproteins. These are later transformed into insoluble proteins by enzymatic removal of their carbohydrate groups. Within 3 or 4 hours of its formation the pellicle becomes colonized with bacteria that are part of the host's indigenous microflora. The bacterial colonization initiates the next stage, called *plaque formation*.

Plaque Formation

Dental plaque is composed mainly of bacteria and their products attached to the tooth surface (Fig. 37-4). Plaque formation does not always result in caries formation; however, smooth surface caries cannot take place in its absence. The chemical and microbiological composition of the plaque changes considerably during its development from a thin,

almost structureless film of material to the more mature, thick, structured form. The primary bacterial species involved in early colonization belong to the genus *Streptococcus*. Microcolonies of bacteria are created, resulting in the formation of distinct ecosystems. The further maturation and development of the ecosystems are subject to environmental factors such as pH, aeration, and the presence of binding agents—calcium, proteins, and glucans, for example.

THE NATURE OF PLAQUE. Chemically, early plaque (1–10 days) consists of approximately 80 percent water, 20 percent glycoproteins, and 1 to 2 percent glucan. As plaque matures, the bacterial populations change. During the first 2–3 days of plaque development the proportion of aerobic species that can be cultivated is greater and the population is primarily coccal. As plaque matures and thickens there is a change in the oxidation-reduction potential that favors the growth and development of the more oxygen-sensitive microorganisms. One sees an increase in species of filamentous organisms such as *Actinomyces* and *Fusobacterium*, and also *Veillonella*. In individuals who are relatively caries free, the predominant streptococcal species recovered from plaque samples is *S. sanguis*. In caries-susceptible persons, however, *S. mutans* and *S. sanguis* can be found in plaque associated with carious lesions.

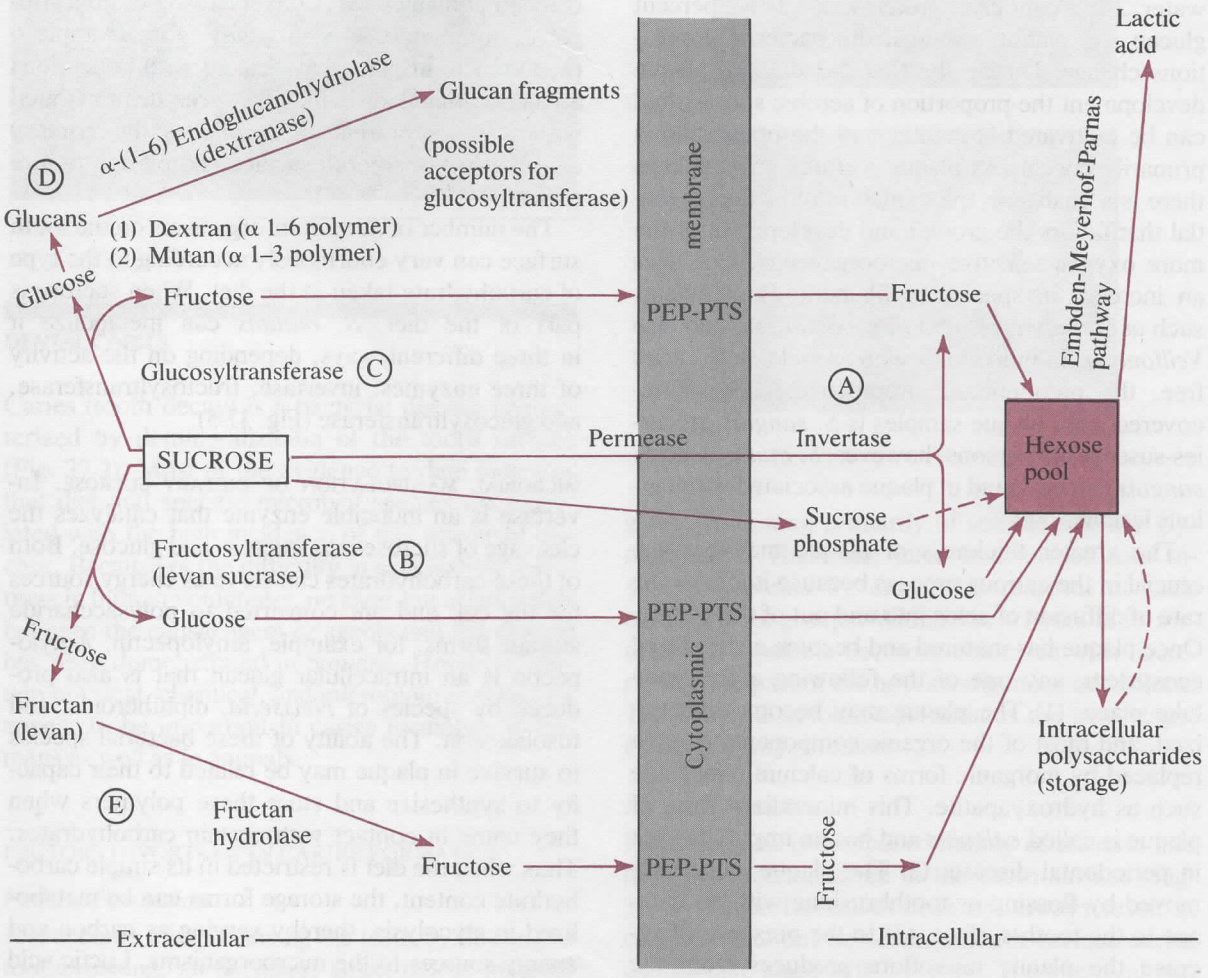
The greater thickness of plaque may become crucial in the carious process because it affects the rate of diffusion of acids into and out of the plaque. Once plaque has matured and become a structured ecosystem, any one of the following events may take place: (1) The plaque may become mineralized, and most of the organic components may be replaced by inorganic forms of calcium phosphate such as hydroxyapatite. This mineralized form of plaque is called *calculus* and has an important part in periodontal disease. (2) The plaque can be removed by flossing or toothbrushing with no damage to the tooth surface. (3) In the presence of sucrose the plaque microflora produces from the catabolism of the sugar acids that may result in the demineralization of the tooth surface and the formation of carious lesions. The pH at which demineralization occurs is around 5.6. The development of a carious lesion in plaque is dependent on many factors. At one time it was believed that the thickness of the plaque was the major contributing factor to the development of caries. It has now been established that the microbial composition of the plaque as well as specific nutritional factors play the most important roles in this pandemic disease.

THE ROLE OF *STREPTOCOCCUS MUTANS*. Because it constitutes a large proportion of the plaque microflora of carious lesions, *S. mutans* is strongly implicated in human caries. Conversely, other noncariogenic streptococci—*S. sanguis*, for example—predominate in plaque associated with noncarious areas. In animal systems it has been demonstrated without a doubt that *S. mutans* is the primary causal agent of smooth surface and pit and fissure caries.

The number of *S. mutans* organisms on the tooth surface can vary enormously according to the type of carbohydrate taken in the diet. When sucrose is part of the diet, *S. mutans* can metabolize it in three different ways, depending on the activity of three enzymes: invertase, fructosyltransferase, and glucosyltransferase (Fig. 37-5).

MICROBIAL MANIPULATION OF DIETARY SUCROSE. Invertase is an inducible enzyme that catalyzes the cleavage of sucrose into fructose and glucose. Both of these carbohydrates can serve as energy sources for the cell and are converted to polysaccharide storage forms, for example, amylopectin. Amylopectin is an intracellular glucan that is also produced by species of *Neisseria*, diphtheroids, and fusobacteria. The ability of these bacterial species to survive in plaque may be related to their capacity to synthesize and store these polymers when they come in contact with certain carbohydrates. Thus when the diet is restricted in its simple carbohydrate content, the storage forms can be metabolized in glycolysis, thereby serving as carbon and energy sources to the microorganisms. Lactic acid is produced, and the acid pH of plaque is maintained.

Fig. 37-5. Sucrose metabolism in *S. mutans*. Glucose, fructose, and sucrose have individually controlled phosphoenolpyruvate-dependent phosphotransferase systems (PEP-PTS). In addition sucrose can be transferred across the cell membrane without phosphorylation by a permease. A. Sucrose transferred across membrane by permease; enzyme invertase cleaves it into glucose and fructose. Both these carbohydrate moieties can enter the hexose pool. Sucrose is phosophorylated in PEP-PTS, but the pathway of sucrose metabolism is unclear (--->). B. The enzyme fructosyltransferase cleaves sucrose into fructose and glucose and polymerizes fructose to form a fructan polymer called levan. Glucose can enter the hexose pool to be converted to lactic acid via the Embden-Meyerhof-Parnas pathway or to be used for intracellular polysaccharide synthesis. C. The enzyme glucosyltransferase cleaves sucrose into fructose and glucose and uses glucose for the synthesis of glucan polymers called dextran and mutan. Fructose is free to enter the hexose pool. D. Glucans can be cleaved by a dextranase, which is an endoglucanase and does not release free glucose. *S. mutans*, therefore, cannot grow with dextran or mutan as a sole source of carbon. E. Fructan (levan) may be cleaved by bacterial fructan hydrolase enzymes to yield fructose, which is then free to enter the hexose pool. (Courtesy J. M. Tanzer.)



Fructosyltransferase (levan sucrase) also cleaves sucrose, but only the glucose moiety is available for catabolism and thus enters the hexose pool (Fig. 37-5). The fructose moiety is used for the synthesis of fructan, which is a beta 2–6–linked polymer of fructose also known as levan. Levan can be found in plaque and is produced by species of streptococci other than *S. mutans* as well as species of *Actinomyces*. Levan is an extracellular polysaccharide and is hydrolyzable by other plaque bacteria, thus serving as an alternate carbon and energy source during periods of low dietary sucrose intake.

Glucosyltransferase is a constitutive enzyme (not subject to induction or repression, but produced continuously). It too catalyzes the cleavage of sucrose. The resulting fructose moiety is released to be used by the cell for energy production, while the glucose moiety is used in the synthesis of a high molecular weight glucan.

Glucans containing predominantly alpha 1–6 linkages are called *dextrans*; those containing predominantly alpha 1–3 linkages are called *mutans*. Both polymers exhibit variously linked branches. Mutans are the least soluble of the glucans and are not easily hydrolyzed by the cell or other plaque bacteria. These "sticky" glucans bring about the aggregation of bacteria and their attachment to the tooth surface. In addition, they form part of the matrix of plaque.

Diet is probably the single most important factor in caries formation. The more sucrose taken in the diet, the larger the amount of plaque formed and the greater the susceptibility to caries formation. Several studies have demonstrated the relationship of dietary sucrose to dental caries formation.

1. When the teeth of rats or hamsters were previously cleaned and all plaque was removed, a diet containing glucose as the only carbohydrate source resulted in the formation of a thin structureless plaque that did not greatly increase in size. On the glucose diet *S. mutans* was barely detectable, and caries activity was reduced. When the diet was changed to one containing high concentrations of sucrose, plaque formation increased rapidly and there was a concomitant rise in the numbers of *S. mutans* and lactobacilli.
2. Caries-inducing streptococci such as *S. mutans* were implanted in the mouths of hamsters as well as humans. The implantation was successful only when the diet contained large amounts of sucrose. Under these conditions the number of lactobacilli as well as *S. mutans* rose. The lactobacilli, which require a low pH for growth (aciduric), may act as secondary pathogens aiding in the demineralization of tooth enamel.

HOST FACTORS IN CARIES OR PLAQUE CONTROL

No direct relationship has yet been established experimentally between the rate of saliva flow and extremes of caries activity. It is fairly well documented, however, that individuals in whom there is a decreased salivary flow or in whom the salivary glands are not functioning exhibit an increased incidence of caries. Saliva, as has already been mentioned, contains glycoproteins that can aid in the formation of dental plaque. But it also has components that affect plaque in such ways as to help reduce the incidence of caries: (1) The mechanical cleansing by saliva prevents the accumulation of plaque. (2) If the concentrations of salivary calcium and phosphate are high enough, they can reduce the solubility of calcium and phosphate in the enamel. (3) The carbonic anhydrase–bicarbonate system of saliva acts as a buffering mechanism against acids produced in plaque. (4) A localized immune system is present in saliva in the form of immunoglobulins, principally secretory IgA.

Even though there is no blood supply to tooth enamel, antibodies in the saliva in the form of sIgA have access to the tooth surface. In humans, the relationship between antibodies in serum and antibodies in saliva with respect to caries incidence is confusing. Some studies show that in caries-resistant individuals there are elevated concentrations of sIgA compared to the caries-susceptible group. In some studies the opposite results are cited. The sIgA may interfere with the adherence of microor-

ganisms in dental plaque, as was discussed earlier. Two immunogens, *S. mutans* and glucosyltransferase, have been used to elevate antibody levels in the oral cavity of germ-free rats. Preparations of whole or broken *S. mutans* cells, when used for local immunization in the oral cavity, gave rise to sIgA primarily and reduced the incidence of caries after infection with cariogenic *S. mutans*. Soluble glucosyltransferase in various states of purification, when used as an immunogen, gave rise to sIgA and serum IgG. These antibodies inhibit the function of glucosyltransferase and thus interfere with one of the mechanisms of aggregation and adherence of microorganisms in plaque.

Studies using primate models of caries have found that protection correlated only with the titer of serum IgG and not with the sIgA titer. The IgG antibodies contact the tooth surface and plaque as a component of the gingival crevice exudate. Furthermore, glucosyltransferase was not found to be an effective immunogen in primates, and vaccines containing *S. mutans* cell walls induced antibody cross-reactive with heart tissue, a situation analogous to rheumatic fever. While a human caries vaccine is possible, considerable effort will be necessary to identify immunogens that are both protective and safe and to develop a regimen for administration.

TREATMENT AND CONTROL

Sucrose Substitutes

It is generally accepted that the reduction of refined sugars such as sucrose in the diet greatly reduces the incidence of caries in humans. Many studies have examined the effect of certain sucrose substitutes as sweeteners in candies, gums, and other edible products. The principal substitutes have been sorbitol, xylitol, saccharin, and aspartame. Sorbitol is a six-carbon sugar found in some fruits. It is used as a sweetener in some candies and gums. It is fermented by *S. mutans*, but so slowly that the pH of plaque does not drop to a point at which demineralization can occur. Reports indicate that sorbitol substitution can significantly reduce the incidence of caries. Xylitol is a five-carbon sugar found in fruits and some vegetables. In one study a group using xylitol-containing products showed a dramatic decrease in caries compared with the control group, whose diet contained foods sweetened with sucrose. Both sorbitol and xylitol can cause diarrhea when used in excess, and xylitol has been implicated as a possible carcinogen.

Saccharin is a nonnutritive artificial sweetener of considerable popularity because of its convenience for consumer use. At one time saccharin was suspected to be a carcinogen. However, extensive epidemiological surveys showed no relationship between its use and human cancer.

Aspartame, a methyl ester of the amino acids aspartic acid and phenylalanine, has recently been approved for human use. While not a universal substitute for sucrose aspartame is finding widespread use and acceptance in a variety of diet and sugarless foods.

Fluoridation

Fluoridation of water supplies (1 to 2 parts per million) significantly reduces dental caries. Controlled studies have demonstrated that caries reduction approximates 50 percent when fluoridated water is drunk during the early years of tooth development. Many studies have also shown that topical application of fluorides (225 to 12,000 parts per million) reduces the occurrence of caries in children by 50 percent. A single application of stannous fluoride per year is apparently as effective as the four applications suggested when sodium fluoride is applied. When stannous fluoride is combined with phosphoric acid (1.23% fluoride in molar orthophosphoric acid) and applied topically to children's teeth, caries reduction approaches 70 percent. High fluoride concentrations applied to the tooth surfaces are bactericidal for *S. mutans* but not *S. sanguis*. At lower concentration fluoride ions exchange with hydroxyl ion, resulting in fluorapatite formation. There is also formation and precipitation of calcium and magnesium fluorides in enamel. These processes result in tooth structure more resistant to acid mineralization. Fluoride also inhibits synthesis of the intracellular storage polysaccha-

ride and interferes with glycolysis, reducing acid formation.

Antimicrobial Agents

Since caries is apparently associated with a microbial agent, many investigators have studied the effects of antimicrobial drugs on caries. Antibiotics such as penicillin, vancomycin, and kanamycin have been used in mouthwashes to inhibit gram-positive acidogenic microorganisms in the oral cavity. The use of antibiotics is contraindicated because of the possibility of superinfection.

Chlorhexidine, used in mouthwashes and topical preparations, is effective as an antimicrobial against many oral microorganisms. It inhibits the development of plaque and aids in removal of already developed plaque. Studies with animals and humans show that chlorhexidine is poorly absorbed and nontoxic, can reduce the oral microflora by 80 to 90 percent with a few rinses, and does not appear to select for drug-resistant strains after prolonged use. The most important aspect of this drug is its ability to adhere to the hydroxyapatite of the enamel as well as the pellicle. The adsorbed drug is released from these sites for several hours, thereby preventing bacterial colonization. Frequent use of chlorhexidine, however, causes discoloration of the teeth and dorsum of the tongue.

The requirement for glucans for attachment of *S. mutans* to the tooth surface has prompted investigators to look for enzymes that could attack the polysaccharides. Fungal dextranases have been tested in mouthwashes, in diet, and in drinking water. They have proved effective in animal studies, but in humans the results are inconclusive. One of the major problems is the variety of branched glucans produced by human oral strains of bacteria and their relative resistance to hydrolysis by many dextranases.

All of the agents mentioned may to some degree aid in the prevention of caries, but none is capable of complete control. To date the best methods for preventing caries are restriction of foods containing high concentrations of refined carbohydrates such as sucrose, fluoride treatment for developing teeth, and mechanical removal of plaque by daily oral hygiene.

Occlusal Sealants

Various sealants may be applied to the occlusal (chewing) surfaces of teeth to establish a physical barrier between the tooth and demineralizing acids of bacterial origin. Sealants are effective in reducing caries development on these surfaces, but their application may be too costly at this time to gain widespread acceptance.

PERIODONTAL DISEASE

Periodontal disease involves the soft and hard tissues surrounding and supporting the teeth. In the adult population periodontal inflammation results in the loss of the fiber attachment of the tooth and the eventual resorption of the supporting alveolar bone. As a result periodontal disease is the major cause of tooth loss. Most investigators today believe that bacteria play a major role in periodontal disease. In recent years some of the bacterial species associated with periodontal diseases have been isolated and characterized. There is also a growing appreciation of the role of host immune responses to periodontopathic bacteria. Immune responses limit the destructive activity of these organisms but at the same time contribute to the disease process. In general, there are three pathological conditions that may exist in the periodontium: (1) gingivitis, or inflammation of the gingivae (gums); (2) periodontitis, a chronic infection that may attack the gingivae but is primarily involved with the periodontal ligament, where infection may lead to pocket formation and alveolar bone loss; and (3) acute necrotizing ulcerative gingivitis, a disease characterized by necrosis of the interdental gingival tissue. Gingivitis and periodontitis have a very high prevalence in most parts of the world, affecting most of the adult population.

FACTORS IMPORTANT IN PERIODONTAL DISEASE

There is a direct correlation between the accumulation of bacterial plaque in the gingival crevice and gingivitis. As many as 2×10^{11} bacteria per gram wet weight of plaque have been recovered from the gingival crevice. Plaque accumulation is often fostered by calculus deposits, but overhanging margins of restorations or food impaction and retention also contribute to its buildup. A favorable prognosis in gingivitis depends on the removal of calculus and the ability to control plaque accumulation. The calcification of dental plaque is better understood today as a microbial process. A number of plaque microorganisms have been shown to accumulate calcium phosphate salts in vitro, and they themselves undergo calcification. A frequently mentioned organism in this process is an aerobic actinomycete, *Bacterionema matruchotii*, one of the more numerous filamentous species in plaque. Calculus is believed to be directly related to gingivitis because the chronic mechanical irritation incites inflammation. Removal of subgingival calculus from the cementum surface by scaling is probably the single most important procedure in treatment of periodontal disease.

Inflammation extends to deeper tissues, from the marginal gingivae into the attached gingivae, with prolonged exposure to increasing accumulations of subgingival plaque and calculus. As collagen fibers of the periodontal ligament are destroyed, the gingival crevice deepens. The epithelial attachment migrates apically (toward the root tip) during this process, eventually leading to periodontal pocket formation. This chronic or established inflammatory lesion may persist for years without apparent periodontal involvement. Eventually, however, with increasing pocket depth there is loss of alveolar bone, and subsequent gingival recession makes periodontitis obvious.

Estimates of the number of bacterial species found in subgingival plaque exceed 250. Of these, less than 100 are regularly observed. Recent studies have recognized less than 20 species with a high degree of association with periodontal disease, almost all of which are gram-negative. Cultural studies have demonstrated a distinct microflora or predominating species in gingivitis, periodontal disease, periodontosis, and subsequently ANUG. Some researchers have begun to question whether periodontal disease is an extension of gingivitis or a separate disease. Organisms with recognized periodontopathic activity are listed in Table 37-4.

Table 37-4. Subgingival Microorganisms with Recognized Pathogenicity in Periodontal Diseases

Bacteroides gingivalis
B. intermedius
Capnocytophaga ochracea
C. sputigena
C. gingivalis
Actinobacillus actinomycetemcomitans
Fusobacterium nucleatum
Wolinella recta
Campylobacter sputorum
Selenomonas sputigena
Hemophilus species
Eikenella species
Eubacterium species

Role of Microbial Products

The resident bacteria in the gingival crevice have pathogenic potential but seldom invade underlying tissue. Their pathogenicity appears to be related to the production of enzymes, toxins, and by-products of metabolism. Some of these important products and their effects are as follows:

1. Leukotoxin inhibition of neutrophils. The neutrophil fulfills a major protective function against subgingival microorganisms and is the predominant leukocyte in acute gingival inflammation. Several organisms have been found to produce leukotoxins that kill the neutrophil (*Actinobacillus actinomycetemcomitans*), inhibit chemotaxis of the neutrophil into an inflammatory lesion (*Bacteroides* species, *Capnocytophaga* sp, *Actinobacillus*), and to interfere with phagocytosis or intracellular killing (*Bacteroides* sp). The leukotoxicity of *A. actinomycetemcomitans* and an underlying defect in neutrophil chemotaxis

have been implicated in the etiology of localized juvenile periodontosis.

2. Metabolic wastes. A wide variety of such products are able to damage crevice epithelium and periodontal tissue. Wastes include butyric and propionic acid, hydrogen sulfide, indole, and ammonia. These compounds are toxic to fibroblasts, they reduce collagen synthesis, and they increase the permeability of the oral mucosal epithelium to plaque and microbial antigens.
3. Collagenase. Collagen is a major protein component of periodontal connective tissue and is the substrate for the enzyme collagenase. Collagenase is produced by *Bacteroides gingivalis* and *B. intermedius*. Few other microorganisms present in the gingival crevice have the potential to hydrolyze collagen.
4. Proteolytic and other enzymes. Many of the periodontopathogens listed in Table 37-4 produce a wide variety of enzymes, among which are protease, phospholipase, and phosphatase. Experimental abscess formation using these organisms has given support to a role for these enzymes in tissue destruction. Of particular importance may be proteases that degrade immunoglobulins G and A.
5. Endotoxin. Endotoxin originating from gram-negative microorganisms increases with increasing gingival inflammation. The lipopolysaccharides of several of the species listed in Table 37-2 have been demonstrated in experimental animal model systems to be capable of inducing inflammation, phagocytosis, bone resorption, and degranulation of mast cells; of activating the alternative complement pathway with release of biologically active cleavage products; and of stimulating the release of lysosomal enzymes from inflammatory cells in periodontal tissue.

Role of the Host

The objective of host defenses is to limit the ability of bacteria to colonize and damage gingival and periodontal tissues. However, the immune response to periodontopathic microorganisms of their products also leads to nonspecific destruction of connective tissue and alveolar bone. The release of hydrolytic lysosomal enzymes from neutrophils, the release of lymphokines from lymphocytes, and the release of collagenase from macrophages are of particular importance. Lymphokines include macrophage activating factor, which attracts macrophages into the inflamed periodontium and enhances their activity. Increased synthesis and release of collagenase from activated macrophages account for extensive connective tissue injury in chronic inflammation. Osteoclast activating factor is another lymphokine produced by both T and B lymphocytes that stimulates osteoclasts to resorb alveolar bone. After elimination of the inciting inflammatory agents, the inflammatory process subsides and tissue repair begins. Successful elimination may require intensive plaque and calculus removal by scaling and possibly antibiotic therapy to bring the subgingival microflora under control.

ACUTE NECROTIZING ULCERATIVE GINGIVITIS

ANUG can be distinguished from plaque-associated gingivitis in that ANUG is characterized by tissue necrosis and microbial invasion of the gingivae, particularly of the interdental papillae. ANUG is caused by bacteria, as has been established by the fact that the disease is susceptible to the action of antibiotics such as penicillin. Of considerable importance may be local predisposing factors and altered host resistance including emotional and physical stress, for example, among troops in combat (trench mouth) or college students during final exams. The disease is characterized by the presence of a "pseudomembrane," which covers the necrotized tissue of the marginal gingiva. Pain and bleeding are also associated with the ulcerated interdental papillae. Smears from the ulcerative tissue show a large number of spirochetes, fusobacteria, and neutrophils. If left untreated, this condition could progress into periodontitis.

SUMMARY

The oral microflora develops into a very complex and dense population in several ecological niches, especially enamel surfaces and the gingival sulcus. Estimates of the number of microbial species colonizing the human oral cavity approach 300. The number of microorganisms in dental plaque and the gingival sulcus exceeds 100 billion per gram wet weight. These are among the most densely colonized sites of the body.

The oral cavity is sterile at birth, but a microflora is quickly acquired by contamination as the fetus passes through the birth canal or by contact with other humans. Some of these microorganisms become permanent residents, and others are present only transiently. Streptococci, especially *S. salivarius*, as well as neisseriae, staphylococci, and veillonellae are among the early permanent colonizers. The gingival sulcus is formed with the eruption of teeth, and sites are thus created that can support obligate anaerobes (the sulcus) and microorganisms with the ability to adhere to hard enamel surfaces. Anaerobes include *Actinomyces, Bacteroides, Fusobacterium, Leptotrichia*, and some diphtheroids. *Streptococcus mutans*, probably the most cariogenic microorganisms for humans, and *S. sanguis* are capable of attaching to the acquired pellicle coating enamel surfaces, and they synthesize large amounts of sticky extracellular polysaccharides from dietary sucrose.

Tooth decay in humans is related to many factors, including salivary flow and diet. In individuals susceptible to dental caries the carbohydrate concentration in the diet, particularly sucrose, is the single most important factor in caries formation. Continued presence of sucrose in the diet promotes the selection of cariogenic microorganisms such as *S. mutans*. *S. mutans* produces glucans and fructans. Glucans cause the aggregation of *S. mutans* cells and are responsible for their irreversible attachment to the pellicle of salivary glycoproteins on tooth surfaces. The accumulation of the bacterial debris on the tooth surface results in the formation of plaque and calculus (mineralized plaque). *Streptococcus* species trapped in the plaque produce lactic acid from the catabolism of sucrose. A drop in pH to below 6 favors the growth and development of *Lactobacillus* species. When the pH of the plaque reaches 5.6, demineralization of the tooth enamel takes place. Heavy plaque accumulation favors the prolongation of demineralizing conditions.

Efforts to prevent dental caries focus on the reduction of plaque accumulation by limiting dietary sucrose intake and use of substitute sweeteners and on plaque removal by regular dental hygiene. Also, teeth can be made more resistant to acid demineralization by the use of fluorides and application of occlusal sealants. Research is continuing in the development of an anticaries vaccine directed against *S. mutans* to stimulate protective levels of secretory IgA antibody in saliva.

Gingivitis is an inflammatory condition of the marginal gingiva brought about by accumulating plaque and calculus. Calculus is believed to be directly involved in gingivitis because the chronic mechanical irritation incites inflammation. Most adults experience gingivitis to some extent.

Periodontitis is an inflammatory condition of the soft and hard tissues surrounding and supporting the teeth. Continued periodontal inflammation eventually leads to resorption of the supporting alveolar bone and tooth loss. Tissue damage is initiated by accumulations of microorganisms and their metabolic products in the sulcus. There is little actual invasion of host tissue by microorganisms. Metabolic acids, indole, and other wastes damage tissue, and enzymes such as collagenase degrade the fiber attachment of the tooth. These processes lead to formation of periodontal pockets. Other microbial products such as endotoxin induce and enhance host inflammatory and immune responses. Although the objective of the immune response is to limit microbial colonization and damage to periodontal tissue it is also involved in the nonspecific destruction of connective tissue and alveolar bone. Neutrophils release hydrolytic enzymes, macrophages release collagenase, and lymphocytes release a variety of lymphokines. The lymphokines include osteoclast activating factor, which stimu-

lates osteoclast cells to resorb alveolar bone.

Only recently have methods permitting more extensive isolation and characterization of microorganisms associated with periodontitis become available. The genera *Bacteroides, Capnocytophaga, Actinobacillus*, and *Fusobacterium* are often cited as important periodontopathogens. Treatment of periodontitis involves scaling to remove plaque and calculus and possibly antibiotics to control sulcus bacteria while tissues heal.

QUESTIONS FOR STUDY

1. Which genera of microorganisms are among the first to colonize the oral cavity of the newborn?
2. List six genera of obligate anaerobic bacteria commonly found in the gingival crevice.
3. Which group of microorganisms comprise the predominant microflora of the tongue?
4. List in general terms factors that may influence the microbial composition of a particular oral site.
5. Name and describe two examples of microbial interactions.
6. Describe the adherence of *S. mutans* to enamel surfaces.
7. Discuss the role of the following in plaque formation: acquired pellicle, extracellular polysaccharides, intracellular storage polysaccharides.
8. Name and describe in general terms the substrate and products of the enzymes glucosyltransferase and fructosyltransferase.
9. Describe the hypothesized antibacterial effect of sIgA.
10. Discuss the role of the lactobacilli in caries.
11. The actinomycetes are most often associated with which oral diseases?
12. Discuss the role of the black-pigmented *Bacteroides* in periodontal disease. How does this group of anaerobic, gram-negative coccobacilli damage tissue?
13. Oral spirochetes are classified in which two genera?
14. How does plaque physically contribute to caries formation?
15. List and discuss the various approaches to controlling plaque formation and caries development.
16. What is the approximate concentration of microorganisms in the gingival crevice?
17. What is calculus, and how does it form?
18. List and discuss microbial products that contribute to periodontopathic activity.
19. Name at least five genera of microorganisms that are strongly implicated in periodontitis.
20. Briefly describe the role of the host's lymphocytes and macrophages in the nonspecific tissue destruction in periodontitis.

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38. HOSPITAL-ACQUIRED DISEASES

OBJECTIVES

To outline the various ways in which hospital-associated diseases can be acquired

To list the sites of infection in the host and the microbial agents most frequently isolated from them

To list those predisposing factors that contribute to hospital-associated infections

To understand the relationship of antibiotic use and hospital-associated infections

To list those hospital procedures that contribute to hospital-associated infections

To outline the techniques and practices that help to prevent hospital-associated infections

Epidemiology
- Transmission
  - Direct Contact
  - Contaminated Fomites and Fluids
  - Airborne Spread
- Nosocomial Infection Rates and Agents Involved
- Factors Important in Nosocomial Infections
  - Microbial Factors
  - Immunological State of the Host
    - Age
    - Metabolic Disorders
    - Immunosuppressive Drugs
    - Trauma
  - Hospital Procedures
    - Surgery
    - Catheterization
    - Antibiotic Chemotherapy
    - Hypodermic Injections
    - Diagnostic Procedures
- Prevention and Control of Nosocomial Infections
  - Environmental Surveillance
  - Hygienic Practices
  - Hospital Personnel Surveillance
  - Patient Surveillance

Infections acquired in the hospital are referred to as *nosocomial* (Greek: *nosos*, disease; *komeion*, to take care of). The epidemiology of hospital-associated diseases is considerably different from that of diseases acquired in the community. In addition, because the patient is already compromised by some underlying condition, hospital-acquired disease may be life threatening. One of the single most important factors contributing to nosocomial disease is the antimicrobial resistance of the microorganisms indigenous to the hospital environment. The increased demand for and use of antibiotics as well as the ability of microorganisms to transfer resistance has led to the selection of antimicrobial-resistant strains that may be infrequently found outside the hospital environment. The proper care, management, and treatment of hospitalized patients to reduce nosocomial disease is an important aspect of the health professional's education.

Our purpose in this chapter is to discuss the factors that contribute to hospital-associated diseases and how they may be prevented.

EPIDEMIOLOGY

TRANSMISSION

The percentage of nosocomial infections acquired from community sources is not easily established in the hospital setting. Infectious microorganisms may reach the patient by direct contact with other patients and hospital personnel, contact with contaminated fomites, or contact with contaminated air.

Direct Contact

Upon entry into the hospital, individuals who are considered infectious must be isolated. This first precautionary measure reduces cross infection between patients. Direct contact between hospital personnel and the patient, however, remains a major avenue of infection, with transmission by hands the most important route for transfer of the infectious agent. For example, a physician removing a dressing from a bacterially contaminated wound could accidentally contaminate his or her hands with infectious microorganisms and transfer them to other patients.

Infections may also be caused by microorganisms of low virulence that are members of one's own microbial flora (endogenous). In endogenous infections the virulence of the pathogen is not as important as the number of microorganisms present in the host and the physiological state of the host. The microbial flora of the host is composed of microorganisms in dynamic equilibrium with the host as well as with other microorganisms. For example, *Escherichia coli* is considered a nonpathogen or an opportunistic pathogen. It exists in equi-

librium with other residents in the gut as well as the host tissue. There is no dramatic increase in the numbers of *E. coli* and no pathological response of the host to its presence. *E. coli* displaced into the genitourinary tract, however, finds itself in a new environment where ecological and physiological pressures may not be exerted on it. Depending on the site of infection and the immunological response of the host, *E. coli* can become highly pathogenic. Endogenous infections are often the result of surgical manipulation, chemotherapeutic treatment, and diagnostic or therapeutic procedures (iatrogenic infections). Frequently in these procedures microorganisms are accidentally displaced from their normal habitat into other areas of the host.

Contaminated Fomites and Fluids

The contact of any instrument with open wounds, mucous membranes, or internal organs of the body represents a mechanism for transmission of infectious agents to the patient. Various solutions or fluids such as intravenous fluids, drugs, blood, or blood products may also be contaminated either by the distributor or by the hospital pharmacy (see boxed essay, p. 892). In other words, a common vehicle may be the source of disease and can result in hospital epidemics. Most infections caused by contact with inanimate objects are incurred during various hospital procedures such as surgery, catheterization, hypodermic injection, and diagnostic procedures.

Airborne Spread

Air is a major vehicle for the dissemination of microorganisms. Usually the airborne microbes are derived from nasal carriers or from the desquamated epithelium of patients and personnel. Individuals with upper respiratory tract infections, or asymptomatic carriers, can infect others by means of discharged air droplets that carry potentially pathogenic microorganisms. These contaminated mucous discharges can make direct contact with the patient, or they may settle on various surfaces to remain infectious for anyone coming in contact with them. Aerosolized bacteria can also be generated by inanimate disseminators. Many cases of pneumonia, for example, have been traced to the use of inhalation equipment. Such instruments are used to generate aerosols of medication for patients with respiratory problems. If equipment or medication becomes contaminated, the nebulizer in the unit will generate bacterial aerosols that are inhaled by the patient.

Microorganisms on the skin have been found on desquamated epithelium, which is readily shed onto bedclothes and thus dispersed into the air on bed making. Individuals with skin diseases such as eczema are prolific disseminators of skin microorganisms because they have more skin scales and because the smaller size of the scales facilitates their transmission.

Microorganisms dispersed into the air from any source can be acquired by the patient through inhalation, by deposition on the skin, or indirectly by contaminated fomites. In the hospital airborne infections are therefore related to the kind of disseminator in operation, the type of filtration system, and the velocity of air and airflow patterns in the hospital.

NOSOCOMIAL INFECTION RATES AND AGENTS INVOLVED

Data have been collected from hospitals in the United States to determine the rates of infection for hospital services as well as the agents implicated in the infection. The isolation rates of the major pathogens from 82 hospitals in 1979 are shown in Figure 38-1. The important finding from this study is the large number of gram-negative species that are involved. In the 1950s the same study would have shown gram-positive species to be the predominant isolates. The discovery and subsequent use of antibiotics that are more effective on gram-negative species—for example, the aminoglycosides—have caused the shift in the type of isolate encountered in disease. This shift is due to the increase of bacterial strains that have become resistant to the aminoglycosides.

PSEUDOMONAS AERUGINOSA PERITONITIS ATTRIBUTED TO CONTAMINATED IODOPHOR SOLUTION—GEORGIA

Five infections involving patients at a hospital have been attributed to use of contaminated Prepodyne Solution. Intrinsic contamination of this iodophor product with *Pseudomonas aeruginosa* has been confirmed by the Centers for Disease Control.

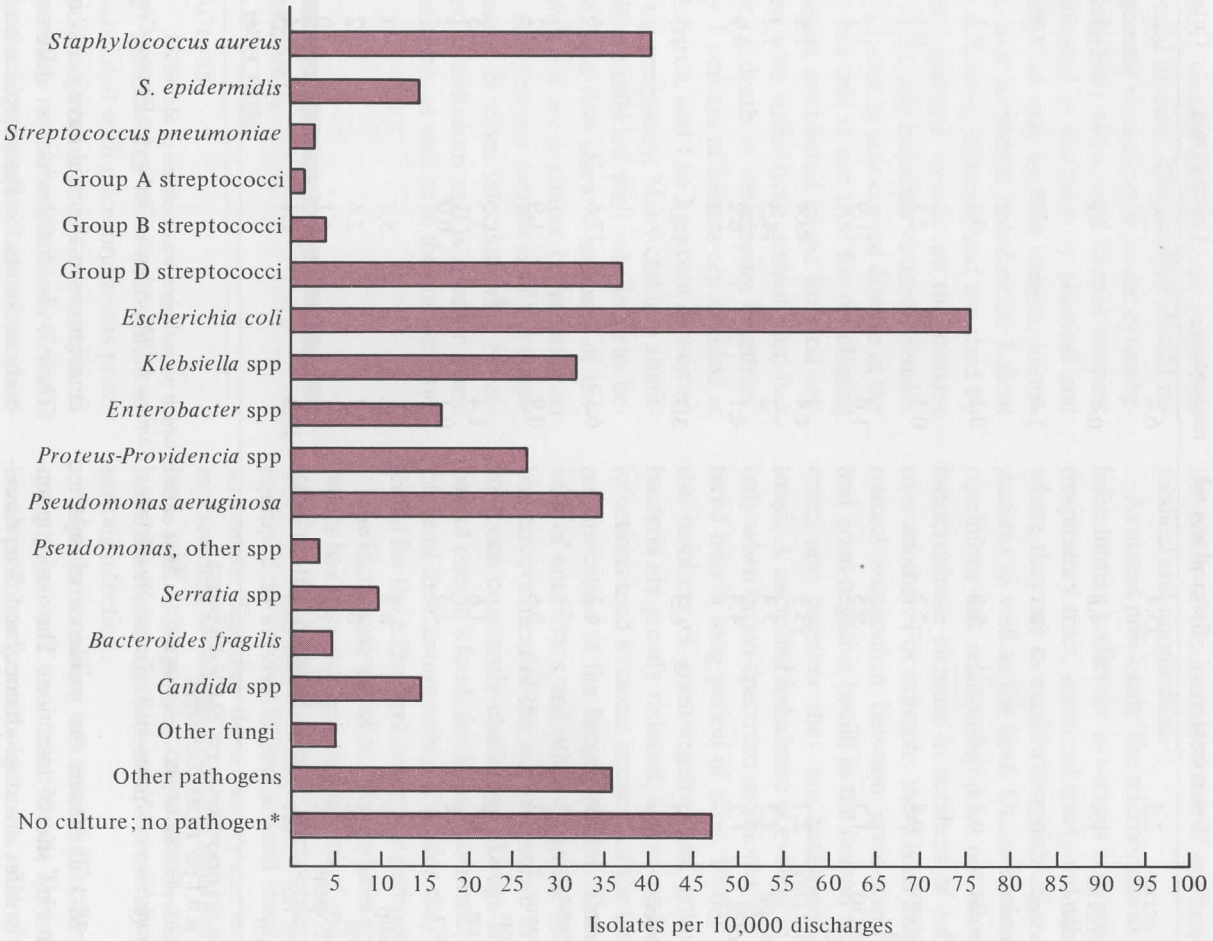
In the period March 9 through April 12, 1982, five chronic peritoneal dialysis patients at a municipal hospital in Atlanta became infected with *P. aeruginosa*. Four patients developed peritonitis, with low-grade fever, cloudy peritoneal fluid, and abdominal pain. Three of these patients used an automatic peritoneal dialysis machine; one used only a bottle cycling machine. All patients had permanent indwelling peritoneal catheters, which were wiped with a 4 × 4 gauze soaked with iodophor, Prepodyne Solution, each time the catheter was connected to or disconnected from machine tubing. Aliquots of Prepodyne Solution were transferred from stock bottles to smaller in-use bottles.

Infection-control personnel at the hospital obtained cultures from the dialysate concentrate, internal areas of the dialysis machines, and a small in-use plastic container that had been filled with Prepodyne Solution. All cultures were negative except that of the Prepodyne Solution, which yielded a pure culture of *P. aeruginosa*. Subsequently, two of eight unopened 1-gallon containers of Prepodyne Solution, one obtained from the dialysis center and a second from another hospital area, were culture positive; they, too, yielded a pure culture of *P. aeruginosa*. The antimicrobial susceptibility patterns of the isolates obtained from the Prepodyne Solution were identical to those of two available isolates from patients; these organisms were sensitive when tested in the hospital to amikacin, carbenicillin, gentamicin, kanamycin, tetracycline, tobramycin, and trimethoprim-sulfamethoxazole, and were resistant to ampicillin and cephalothin.

Poloxamer-iodine and povidone-iodine are the most commonly used iodophor preparations in hospitals; both preparations have now been demonstrated to be vulnerable to intrinsic contamination. Although several iodophors (antiseptic preparations of which Prepodyne Solution is one) are widely used in hospitals for disinfectant purposes, they are FDA approved only for antisepsis of skin and mucous membranes.

From *MMWR* 31:197, 1982.

Fig. 38-1. Isolation rates of selected pathogens that cause nosocomial infections, 1979. Rate is the number of isolates per 10,000 discharges; up to four isolates may be reported per infection. (From *National Nosocomial Infection Study: Annual Summary, 1979*. Atlanta: Centers for Disease Control, 1982.)



*Includes infection for which no culture was obtained or from which no pathogen was isolated from a single site.

Table 38-1. Rate[a] of Selected Pathogens by Site of Infection, 1979

Agent	Primary bacteremia	Surgical wound	Lower respiratory	Urinary tract	Cutaneous	Other
Staphylococcus aureus	2.5	15.4	6.4	2.9	8.0	6.0
Staphylococcus epidermidis	1.4	4.8	0.4	4.9	1.7	2.5
Streptococcus pneumoniae	0.2	0.1	2.0	0.0[b]	0.0[b]	0.3
Streptococcus group A	0.1	0.6	0.1	0.1	0.2	0.4
Streptococcus group B	0.4	2.2	0.5	2.1	0.3	0.9
Streptococcus group D	1.3	9.5	1.0	21.9	1.7	2.8
Escherichia coli	2.7	13.9	4.4	49.4	1.9	3.9
Klebsiella spp	2.5	5.4	7.2	14.5	1.2	2.2
Enterobacter spp	1.2	4.4	4.1	6.2	0.9	1.1
Proteus-Providencia spp	0.4	6.3	3.3	14.3	1.3	1.8
Pseudomonas aeruginosa	0.9	6.1	6.1	17.6	1.6	2.7
Pseudomonas other spp	0.3	0.6	0.9	1.9	0.2	0.3
Serratia spp	0.7	1.6	2.4	3.2	0.5	0.6
Bacteroides fragilis	0.5	3.3	0.1	0.0[b]	0.2	0.6
Candida spp	0.9	0.8	1.6	5.7	1.3	3.7
Other fungi	0.2	0.3	0.7	2.6	0.2	0.9
Other pathogens	2.2	13.0	7.3	5.1	2.0	5.7
No culture; no pathogen[c]	0.1	15.0	15.8	2.1	3.4	12.0

[a]Number of isolates reported per 10,000 discharges.
[b]Incidence is 0.1 per 10,000 discharges.
[c]Includes infections for which no culture was obtained or from which no pathogen was isolated or identified.
Source: From *National Nosocomial Infection Study: Annual Summary, 1979*. Atlanta: Centers for Disease Control, 1982.

Table 38-1 illustrates the incidence of selected pathogens by site of infection. The major gram-positive isolates are staphylococci and *Streptococcus* group D. *Staphylococcus aureus* is indigenous to about one-third of the population, but in hospital personnel the figure is higher than 70 percent. In these carriers high concentrations of *S. aureus* are found in the nasal cavity and skin and lesser concentrations in the oral cavity and intestinal tract. Their wide distribution on different sites of the body accounts for their implication in a variety of infections. Since *Streptococcus* group D is found in the intestinal tract, it is more frequently associated with surgical wounds and urinary tract infections. When one looks at the gram-negative species, particularly those that are indigenous to the intestinal

tract, one is not surprised to find them involved in urinary tract infections.

Although not included in national nosocomial statistical studies, viruses also play an important role in nosocomial disease. The student should recall that endogenous viruses such as the cytomegalovirus, Epstein-Barr virus, and herpes simplex viruses are activated in the host by physical and chemical factors that may include trauma, immunosuppression, and hormonal imbalances. Latent viruses, when activated, replicate and are shed by the host. Other important viruses are the measles virus, rubella virus, and hepatitis viruses. Measles virus is less of a factor in nosocomial disease in the United States, but this is not true for developing countries. Measles contracted in the hospital setting by children who suffer from malnutrition is a frequent cause of death in developing countries. Approximately 1 percent of infants are infected at birth by rubella virus, and 1 to 3 percent of women shed virus during pregnancy. Many children admitted to hospitals are infected with rubella virus before admission, and few show symptoms of disease. These children are a source of infection to other children and younger members of the hospital staff. Hepatitis B virus infection may be acquired by blood transfusions and is a major cause of disease in hospitals as well as in the community.

FACTORS IMPORTANT IN NOSOCOMIAL INFECTIONS

MICROBIAL FACTORS

Recent findings indicate that most nosocomial infections are associated with microorganisms previously described as opportunistic—for example, *E. coli, Staphylococcus epidermidis*, and *Serratia marcescens*. In the compromised host all microorganisms must be considered potentially pathogenic, partly because changes in medical procedures have brought about changes in the microorganism, its environment, and its disease manifestations. New or unusual microorganisms now appear as permanent residents of the hospital atmosphere, and each must be treated as a potential pathogen. Most microbial changes have been the result of the increased use of antibiotics, both medical and nonmedical.

As stated previously, the microorganisms that inhabit human bodies are in various ecological niches (respiratory tract, intestinal tract, and other sites), where they are in equilibrium with other microorganisms as well as the host. Under normal healthy conditions this relationship does not allow for the indiscriminate increase in numbers of one species over another. For example, there is an independent mutual antagonism between gram-positive cocci and gram-negative bacilli in the human respiratory tract, and together they are antagonistic to the fungi. A microbial imbalance is evident in the host only when broad-spectrum antibiotics are administered over a long period of time. In this situation the numbers of gram-negative and gram-positive bacteria are greatly reduced, and secondary fungal infections tend to occur because of the uninhibited multiplication of the fungal species. Since the advent of antibiotics and other drugs the intermicrobial environment of the microorganism in the host has been constantly challenged. Drugs are now a part of people's food, drink, and cosmetics, and because of their antimicrobial activities they have selected for the infrequent low-grade pathogens.

The increasing use of new procedures and instruments has also changed the pattern of microorganisms in the hospital. Some microorganisms are equipped to survive in and around these new instruments. Together these factors can lead to the replacement of normal microbial inhabitants in the host with infrequently encountered microorganisms. Such altered relationships are likely to lead to serious infection.

Organisms causing nosocomial infections are frequently resistant to antimicrobials. This resistance involves not only microorganisms of low virulence but also the more frequently isolated pathogens such as *Staphylococcus aureus*. As many as 75 percent of hospital personnel can be described as carriers of antimicrobial-resistant *S. aureus*. The resistance of this organism was for many years

confined to the antibiotic penicillin G, but the organism is now resistant to several aminoglycosides as well as methicillin. Gram-negative organisms such as *Serratia, Klebsiella*, and *Pseudomonas* are resistant to a variety of antimicrobials including many of the important aminoglycosides. Still there are some microbial species that have remained uniformly susceptible to specific antimicrobials—for example, group A streptococci, meningococcus, and *Treponema pallidum*, all of which are susceptible to penicillin. In addition the resistant organisms that inhabit the host do not usually persist when the antimicrobial is removed. Despite these positive signs antimicrobial resistance does affect the outcome of infection in the patient.

The two factors leading to increasing prevalence of antimicrobial resistance as a cause of nosocomial infections are the frequent use of antimicrobials and the mechanisms of resistance transfer in microorganisms. Chromosome-mediated resistance is a problem, but most of our concern today is with resistance mediated by plasmid transfer factors and transposons. Resistance transfer occurs not only between members of the same genus and species but between species and within genera. Microorganisms regarded as nonpathogenic can be the source of resistance factors. The most common sites of resistance transfer are the gastrointestinal tract and the skin. Resistance transfer in the bowel does not depend on the affected individual's receiving antimicrobial agents at the time of transfer. Transfer on the skin, however, appears to occur more frequently in the presence of topically applied antimicrobial agents. Because plasmids can carry multiple resistance markers, selection of resistance to one drug used in treatment may be accompanied by resistance to unrelated drugs. Within the hospital environment the selective pressures for survival of the multiply resistant strain are related to frequency of use of each of the drugs. Even when antimicrobial usage is decreased, resistant microorganisms are capable of persisting. The fact that hospital-associated strains are also transferred to those in the community (and vice versa) is of great concern to the medical community. Many examples now exist in which some microorganisms have developed resistance to antimicrobials outside the hospital: *Hemophilus influenzae* to ampicillin, *Salmonella typhimurium* to ampicillin, *Staphylococcus aureus* to methicillin, pneumonoccus to penicillin and tetracyclines, and *Shigella* species to sulfonamides and tetracyclines. The hospitalized patient undergoing drug therapy, therefore, represents a source of multiply resistant microorganisms not only to other hospitalized patients and hospital staff but to the community as well.

IMMUNOLOGICAL STATE OF THE HOST

The single most important factor determining susceptibility to infection is the relative immunological state of the host at the time of infection. The immunological state can be depressed under certain conditions.

Age

In the few months after birth, the child is very susceptible to bacterial infection—especially the premature child. It is well known that the serum of the newborn does not exert the bactericidal activity that the maternal serum does. The infant serum shows little opsonic activity against such gram-negative bacteria as *E. coli* and *S. marcescens*. Diarrhea caused by enteropathogenic *E. coli* is a major affliction of infants in the hospital nursery. It has not been established, however, that this infection is related to the lack of specific gram-negative activity within the serum. Neonatal sepsis occurs more frequently in premature infants, and fatalities are as high as 50 percent. *E. coli* is the most frequent cause of neonatal sepsis. Up to 6 years of age pediatric patients remain highly susceptible to infection with *Hemophilus influenzae*. Their serum is particularly deficient in antibody for this species of bacteria. Recent extensive use of ampicillin in pediatric diseases has triggered the appearance of unresponding, antibiotic-resistant varieties of *H. influenzae*. In consequence, the number of cases of fatal meningitis among infants has risen.

Older patients with cardiovascular disease, urinary tract abnormalities, or respiratory conditions

are especially prone to infections while in the hospital. So are older patients kept alive with immunosuppressive drugs and immunosuppressive procedures. When catheterization, positive pressure apparatus, or anesthesiology equipment is applied to these compromised patients, they are often unable to withstand the challenge of the microbe.

Metabolic Disorders

Patients with leukemia or any hematological disorder that involves the immune system must be considered likely candidates for hospital-acquired infections. In leukemic patients the problem is often compounded because of the need for radiation or drug therapy to reduce the number of leukocytes. This kind of therapy leads to immunological deficiencies.

Diabetics are also highly susceptible to nosocomial infections. Their susceptibility is related to secondary effects of the disease such as renal insufficiency, acidosis, and vascular insufficiency, particularly of the lower extremities. Insulin-dependent patients with diabetes mellitus suffer more frequent and severe staphylococcal infections than nondiabetics.

Immunosuppressive Drugs

Immunosuppressive drugs are used therapeutically for immunological disease in which there is a need to reduce the level of leukocytes in the host system. They are also used prophylactically to prevent the rejection of transplant tissue in the immunologically sensitive recipient. The three classes of immunossuppressive agents are (1) the lymphocytolytic agents such as the corticosteroids; (2) the metabolic analogs, such as azathioprine, which interfere with DNA synthesis of lymphoid tissue; and (3) the alkylating agents, such as nitrogen mustard, which depurinate nucleic acids. The function of these agents is to suppress the cellular or humoral immune systems of the host. Corticosteroids, for example, generally depress the formation of antibodies and suppress the delayed hypersensitivity reaction that is believed to be responsible for transplantation immunity.

In the immunosuppressed state the patient is literally at the mercy of any opportunistic pathogen. In renal transplantation, for instance, infections can be caused by bacteria, fungi, and viruses. Most fatalities of fungal origin are associated with species of *Candida* and *Aspergillus*. Fatal septicemias of bacterial origin are attributed to the gram-negative bacilli, particularly species of *Pseudomonas*. Hepatitis is often the most serious viral complication. Cytomegalovirus (CMV) infection is also a frequent complication in renal transplantation but is seldom fatal. CMV infection is thought to be due to the activation of a latent virus triggered by immunosuppressive therapy.

Trauma

Individuals in accidents that cause trauma are susceptible to infection. Traumatic wounds, by crushing and tearing, produce large amounts of dead fat with poor vascularity. This type of condition lends itself to microbial contamination and sepsis by microorganisms usually considered to be noninvasive. Burn patients, for example, are very susceptible to microbial infection because of the presence of necrotic tissue and the loss of neutrophil function that is associated with burns. Microorganisms associated with burn wound infections are *Pseudomonas aeruginosa, S. aureus*, and *Streptococcus pyogenes*. *P. aeruginosa* and other *Pseudomonas* species thrive in moist environments, and in the hospital they are frequent contaminants of humidifiers and nebulizers. *Pseudomonas* species are also resistant to many antimicrobials and disinfectants. The mortality from *Pseudomonas* septicemia in burn wound patients can be greatly reduced with the administration of a polyvalent vaccine in conjunction with an immunoglobulin prepared from the serum of vaccinated healthy volunteers.

HOSPITAL PROCEDURES

Surgery

Unsterilized instruments, sutures, sponges, and irrigating solutions as well as the surgical team itself are the main sources of infection related to surgery. Only sterile equipment and solutions are used dur-

Table 38-2. Rates of Infection of Various Types of Wounds

Type of wound	Total number of wounds	Number of wounds infected (%)
Clean	47,054	732 (1.5)
Clean-contaminated	9,370	720 (7.7)
Contaminated	4,442	676 (15.2)
Dirty	2,093	832 (35.0)
Total	62,959	2,960 (4.7)

Source: From P. Cruse, *Surg. Clin. North Am.* 60:27, 1980.

ing surgical activities. In addition the surgical team wears sterile gowns, masks, and gloves. The patient has many nonspecific mechanisms for resisting infection. Surgery and related procedures can interfere with these natural resistance factors and may be responsible for severe and sometimes fatal infections. Since tissue is being cut, the normal host defense mechanisms are breached, and the reduction in host resistance is worsened by anesthesia. The number of microorganisms that gain entrance to the traumatized tissue as well as the site of the surgical operation greatly influences the rate of infection. Wounds can be divided into four types: clean, clean-contaminated, contaminated, and dirty (Table 38-2):

1. *Clean wounds* are those in which no hollow muscular organ was opened and no break in aseptic technique occurred.
2. *Clean-contaminated* refers to the opening of a hollow muscular organ with minimal spillage of contents.
3. *Contaminated wounds* refers to the opening of hollow organs with gross spillage of contents.
4. *Dirty* refers to the presence of pus or perforated viscera.

The probability of infection goes up further if the patient has been compromised by immunosuppressive drugs, malnutrition, or an underlying illness. Studies with animals show that within the first 3 hours of bacterial contamination of tissue the host's antimicrobial defenses are at their peak. This 3-hour peak activity can be strengthened through the use of antibiotics. If the peak antimicrobial activity of the antibiotic can be coordinated with the host's, there is a better chance of resisting infection.

Table 38-3. Association of Microbial Infections with Certain Predisposing Factors

Predisposing factor	Microorganisms involved
Burns	*Pseudomonas, Serratia, Staphylococcus*
Urinary catheterization	*Pseudomonas, Proteus, Klebsiella, Serratia, Staphylococcus, Candida*
Intravenous catheterization	*Staphylococcus, Candida, Cryptococcus*
Diabetes	Gram-negative bacilli, *Staphylococcus, Candida*
Abdominal surgery	Anaerobic streptococci, *Serratia, Klebsiella, Proteus, Staphylococcus*
Cardiac surgery	*Staphylococcus, Aspergillus, Candida, Klebsiella*, anaerobic streptococci
Renal transplantation	*Aspergillus, Pseudomonas*, hepatitis virus, cytomegalovirus
Diseases and conditions involving lymph system	*Pseudomonas, Staphylococcus*, any fungi, cytomegalovirus, herpes zoster virus

Most postoperative infections occur when surgery has been performed on the alimentary, respiratory, or genitourinary tract. In recent years the number of postoperative infections caused by gram-negative species, especially species of *Pseudomonas, Klebsiella, Proteus*, and *Serratia*, has increased (Table 38-3). Much of this increase is directly correlated with the prophylactic use of antibiotics. The major problems result from the extended use of antibiotics after surgery. When anti-

biotics are continued longer than 1 week after surgery, the incidence of infection rises dramatically.

In the past 15 years, transplantation of organs and tissues has become a common surgical procedure. The patient receiving the transplant is confronted with a foreign tissue and must be treated with drugs that prevent the immune system from recognizing these foreign tissues. Unfortunately, under the influence of these drugs the immune system may not recognize microorganisms, even the patient's own. In an immunosuppressed state the transplant patient often cannot withstand the microbial challenge, and fatal infections may occur. The more profound changes seen in the immunosuppressed patient take place in the cellular immune system: T cell numbers decrease, and some T cell functions are depressed. These changes are responsible for infections by organisms that are usually controlled by the cell-mediated response. In renal transplantation, for example, infections by fungi and viruses are common and account for up to one-third of the deaths.

Catheterization

Catheterization is a process in which a tubular device (catheter) is inserted into a blood vessel or the urinary tract. Catheters may be used diagnostically or therapeutically. Improper sterilization of the catheter or contamination of sterilized catheters by attending hospital staff represents a potential risk to the patient. The catheter is the single most important predisposing factor in nosocomial infections. Nearly 10 percent of all hospitalized patients receive a urinary catheter. Most catheter-associated infections occur at the time of insertion of the catheter when microorganisms on the body surface are inserted into the urethra. In addition infections occur when the juncture between catheter and collecting tube is broken, permitting microbial contamination. Indwelling catheters are used for the drainage of urine in patients whose urine flow is obstructed. Bacteria can pass into the bladder urine along the catheter tubing by a backward flow of voided urine from a contaminated drainage bottle. The development of the closed sterile drainage system equipped with a nonreturn valve that prevents the reflux of urine from the drainage bag into the drainage tube has markedly reduced the number of urinary tract infections. Despite this development, infections do occur because of the lack of aseptic technique on the part of the hospital personnel. Most infections are caused by the gram-negative bacilli (see Tables 38-1 and 38-3). These infections are increased when the patient is being treated with antibiotics.

Intravenous catheters not inserted aseptically and left in place for more than 48 hours are a source of infection. Infections have also resulted from the application of broad-spectrum antibiotics at the site of catheterization. This topical antibiotic treatment reduces the number of bacteria but increases the fungal population, particularly species of *Candida*. Infections can also occur when the patient's total nutrition comes from intravenous catheterization. Many of the protein hydrolysates used in total parenteral nutrition serve as very selective nutritional sources for species of *Candida*. Fatal septicemias have been caused by this catheterization procedure.

Antibiotic Chemotherapy

Areas within the hospital having the highest usage of antimicrobials are intensive care units, burn units, and special surgery care areas. These areas also have the highest prevalence of antimicrobial-resistant bacteria. Colonization with these antimicrobial-resistant bacteria will occur even in those who are not being treated with antimicrobials. This is related to the individual's time of exposure to patients who are being treated with antimicrobials.

There has been considerable debate concerning the prophylactic use of antimicrobials for surgical techniques. Antimicrobial-resistant microorganisms can arise endogenously if the antimicrobials are improperly used. In surgical operations in which there is no contamination, the administration of antimicrobials is not recommended. The following procedures are currently considered acceptable for antimicrobial prophylaxis:

1. Cardiovascular—valve and open heart, coronary artery bypass

2. Orthopedic—prosthetic joint
3. Intestinal—colonic
4. Biliary tract—patient over 70 years of age, acute cholecystitis, obstructive jaundice, or stones in common bile duct
5. Gynecological—vaginal hysterectomy, caesarian section
6. Urological—patients bacteriuric before urological procedures

The antimicrobial agent should be administered 1–2 hours before surgery, and the last dose (doses should not exceed six) should be administered less than 24 hours after surgery.

Hypodermic Injections

Hypodermic injections can be a source of infection in three ways: (1) use of contaminated needles or syringes, (2) displacement of an infectious agent from the surface of the skin into the bloodstream or underlying tissue in the injection process, and (3) use of contaminated solutions for injection. Of the three, injection of contaminated solutions is the principal source of infection. Any kind of injectable fluid such as blood, vitamins, and vaccines may be contaminated before injection and thereby transmit infectious microorganisms to the patient. Blood transfusions remain a constant means of infecting the compromised patient. In the United States from 20,000 to 30,000 cases of viral hepatitis are reported each year arising from transfused blood acquired from donors infected with the hepatitis virus. Blood donors are now being screened for hepatitis B surface antigen, and whenever possible washed packed red blood cells should be used for transfusions. These procedures alone can reduce the incidence of transfusion hepatitis.

Diagnostic Procedures

Diagnostic procedures or equipment used by physicians or other medical personnel on patients have been implicated as sources of nosocomial infection. The reason is that the devices or instruments are often not decontaminated before use on the next patient. Among the instruments implicated are cystoscopes, urometers, cannulas of urinary catheters, gastrointestinal fiberoptic endoscopes, and pressure-monitoring devices such as venous and arterial pressure transducers.

PREVENTION AND CONTROL OF NOSOCOMIAL INFECTIONS

ENVIRONMENTAL SURVEILLANCE

One of the keys to the management and control of hospital-acquired infections is surveillance. In the past few years it has become evident that antibiotics have not reduced the risk of hospital-associated infections. The only important change brought about by the use of these drugs is a shift in the type of infectious agent involved in infections. Today the gram-negative bacilli have supplanted the gram-positive cocci in the number of hospital-acquired infections. Many hospitals now have resident epidemiologists or infection-control nurses whose duty it is to determine the frequency and kind of nosocomial infections. Attempts to reduce infection by surveillance and control of environmental factors have shown only moderate success. Culturing of the entire hospital environment is not practical and in many instances is of little value. There are procedures that are believed to be of maximum benefit, and these should be performed in all hospitals:

1. Environmental culturing only as part of an investigation of a disease outbreak or other problem of patient infection.
2. Testing the effectiveness of steam and ethylene oxide sterilizers.
3. Testing the sterility of products prepared in the hospital that have been shown to present risk of contamination. These products include infant formula, hyperalimentation fluids prepared in the hospital, and breast milk collected for group use.
4. Testing the effectiveness of decontamination and disinfection of instruments such as endoscopes,

Table 38-4. Useful Disinfectants and Antiseptics Employed in the Hospital

Compound[a]	Activity against: Vegetative bacteria and fungi	TB	Hepatitis virus	Spores	Uses (and limitations)
Alcohols					
Ethyl	+	+	–	–	Skin antiseptic
Isopropyl	+	+	–	–	Skin antiseptic
Iodine					
Tincture of iodine	+	?	–	–	Skin antiseptic
Iodophors	+	+	–	–	Unstable, skin antiseptic
Phenols and cresols					
1–2% Phenols	+	+	–	–	Disinfectant, stable in solution
Chlorophenol	+	+	–	–	More active than phenol but more toxic
Hexachlorophene	+	–	–	–	Antiseptic, good surgical scrub
Chlorhexidine	+	–	–	–	Skin antiseptic
Chlorine					
Chloramine	+	–	–	–	1–2% solution for wounds
Sodium hypochlorite	+	+	+	?	Disinfectant, unstable in solution
Formaldehyde	+	+	+	+	Disinfectant, noxious fumes
Glutaraldehyde	+	+	+	+	Disinfectant, unstable, toxic
Ethylene oxide	+	+	+	+	Disinfectant, toxic, absorbed by porous material
Quaternary ammonium Compounds	+	–	–	–	Antiseptic, ineffective against gram-negatives

[a]0.2% Sodium nitrite added to alcohols, formalin, quaternary ammonium compounds, and iodophors prevents corrosive actions of these agents; 0.5% sodium bicarbonate added to phenolic compounds reduces the corrosive action of those compounds.

inhalation therapy equipment, and physical therapy equipment.

Environmental surveillance is but one of the roles of the infection-control nurse, who must also become involved in control, teaching, and implementation of policy.

HYGIENIC PRACTICES

It is important that housekeeping practices as well as patient-related medical procedures be evaluated. The maintenance of a satisfactory standard of hygienic practices is the duty of all hospital personnel from physician to kitchen cook. Disinfectants and antiseptics are used in the hospital to maintain a standard of hygiene. The use of a particular disinfectant or antiseptic depends on a number of factors (Table 38-4):

1. The nature of the object to be treated and the presence or absence of residual material on the object that might neutralize the effect of the agent, such as soap or other organic material.
2. The type of pathogen to be treated—spores, vegetative bacteria, viruses.
3. The antimicrobial spectrum of the disinfectant or antiseptic.
4. The strength of the agent and the time required for it to exert a bacteriostatic or bactericidal effect.

Hygienic practices are extremely important in

controlling and preventing nosocomial infections. It is necessary to maintain aseptic technique when handling instruments, catheters, intravenous therapy equipment, and any other type of device that may come in contact with the hospitalized patient. The one practice that probably reduces the incidence of nosocomial infection more than any other, and yet is often overlooked, is handwashing. It is estimated that at least 50 percent of hospital infections could be prevented by handwashing. Although cross contamination by hand transmission occurs frequently in the hospitalized patient, handwashing is not consistently performed. The microflora of the skin harbors an indigenous population of microorganisms such as *S. epidermidis* and *Propionibacterium* species, both of which are considered relatively nonvirulent. Because of constant contact with patients and the perpetuation of certain species in the hospital, large numbers of more virulent microorganisms are also carried on the skin—for example, *S. aureus* and gram-negative species. It is very important, therefore, during the handling of patients, particularly when going from one patient to another, that contaminating microorganisms be removed from the skin. Washing hands with soap and water is sufficient to reduce the microbial population of the skin, but because of the drying effect of soap and the failure of soap to remain bacteriostatic for any length of time, other antiseptic agents are more suitable. Iodophors and 3 percent hexachlorophene are two agents that are extensively used today. In 1976 the FDA approved the use of chlorhexidine gluconate (CG) with 4% isopropyl alcohol in a sudsing base (Hibiclens). It has been approved for use as a surgical scrub, patient-care handwashing agent, and superficial wound cleanser. This preparation appears to achieve a more immediate reduction in the skin flora than the other agents and has a more sustained antimicrobial effect.

HOSPITAL PERSONNEL SURVEILLANCE

The immunization records of all hospital personnel should be checked and vaccinations given or recommended if they have not been done. Certain personnel should be vaccinated for tuberculosis and offered immunization against poliomyelitis, tetanus, diphtheria, and rubella. The rubella vaccination is especially important for members of hospital staff who come in contact with nurses, doctors, patients, and pregnant women (see Chapter 34). Every one of these procedures is important not only to hospital personnel but also to the patient who in the compromised condition is most susceptible to infection.

PATIENT SURVEILLANCE

When patients are admitted to the hospital, their medical files should include an infection "report card." During their stay, data regarding disease treatment should be recorded. This information should include

1. The type of infection, if any, the patient had on admission to the hospital
2. The kind and site of any hospital-acquired infection
3. The organism isolated from the infection
4. Any surgical procedure or chemotherapy that preceded the development of the infection
5. The antibiotic regimen employed to combat the infection
6. The length of time required to control the infection

The information gathered from the report card could be compiled each week and a monthly report distributed to the infection control committee. The data would thus include statistics on infections as to site (urinary tract, respiratory tract), causative agents isolated, and antibiotic regimen employed. An increase in the rate of infection could then be investigated and the source of infection uncovered.

If an infectious disease is detected in the patient on admission to the hospital, he or she may require isolation. Isolation is necessary for persons who have such diseases as meningococcal meningitis, staphylococcal pneumonia, tuberculosis, diph-

theria, whooping cough, measles (rubella and rubeola), smallpox, chickenpox, mumps, influenza, or pneumonic plague. Patients who have been compromised by hospital procedures should also be isolated from other patients. This group would include patients recovering from open-heart surgery, transplantation, and burns, and those requiring renal dialysis. It is very important to protect these individuals from airborne microbes that are easily transferred in an open ward. Isolation can be accomplished by means of plastic tents and laminar airflow, in which a positive current of filtered air is directed at the patient. The positive pressure of this airflow prevents access of contaminated air from other sources.

SUMMARY

Hospital-associated infections are called nosocomial. The hospitalized patient is more susceptible to infection because of his or her underlying condition. The microbial agents responsible for nosocomial disease can be transmitted from patient to patient indirectly via hospital personnel, directly by contact with hospital staff, by contact with contaminated fomites or solutions, and by airborne spread. Most nosocomial infections are caused by species that are part of the victim's own microbial flora. The most prevalent microbial groups causing infection are the gram-negative bacilli, which include *Escherichia coli, Pseudomonas aeruginosa*, and species of *Klebsiella, Serratia*, and others. *Staphylococcus aureus* is still the most frequently isolated species, primarily because most hospital personnel are carriers of the microorganism. Microbial factors, host factors, hospital procedures, and trauma all contribute to the acquisition of nosocomial disease.

The microorganisms that are indigenous to the hospital environment are especially equipped to survive there. The frequent use of antimicrobials has selected for specific strains most of which are part of the environment and can inhabit the host. Their ability to cause disease is not necessarily related to their ability to produce toxins but rather to their unrestricted growth in the absence of competitors and the depressed immune response of the host. The increasing prevalence of antimicrobial-resistant hospital strains is due to the frequent use of antimicrobials and the genetic mechanisms used by bacteria to transfer resistance.

The immunological state of the host is the single most important factor determining an individual's susceptibility to infection. Age, metabolic disorders, and use of immunosuppressive drugs reduce one's resistance to infection, particularly by the opportunistic microorganisms. Burn patients and others in whom the muscles and tissue of the body have been traumatized are also prone to infection.

Hospital procedures including surgery and diagnostic and therapeutic techniques lead to the introduction of microorganisms into host tissue. Most of the time the organisms are part of the host's flora that are displaced by the surgical procedure or diagnostic or therapeutic technique. Catheterization, which is performed on many patients, is the single most important procedure that predisposes to infection. Areas of the hospital where antimicrobials are most frequently used have the highest prevalence of antimicrobial-resistant bacteria, which can infect even those who are not receiving antimicrobials. Any type of hypodermic injection can also lead to infection, usually because of displacement of organisms from the host's skin into the deeper tissue or bloodstream. Blood transfusions are frequently the cause of hepatitis.

Surveillance and maintaining hygienic practices are most important in reducing the incidence of nosocomial infections. Environmental sampling, handwashing, and patient and staff surveillance all contribute to better patient care.

QUESTIONS FOR STUDY

1. What is the difference between the epidemiology of hospital-acquired diseases and that of community-acquired diseases?
2. What hospital procedure is most responsible for nosocomial disease?
3. Which microbial species are most frequently associated with disease in burn units? Surgery?
4. Are most nosocomial diseases due to microorganisms acquired endogenously or exogenously? Explain.
5. Why is *S. aureus* implicated in so many nosocomial infections?
6. What host factors contribute to nosocomial infections? Hospital factors?
7. What factors help to maintain microbial antibiotic resistance in hospital-associated microorganisms?
8. Which microorganisms are most frequently associated with neonatal sepsis?
9. What is the difference between a clean wound and a clean-contaminated wound?
10. What is the regimen for antimicrobial prophylaxis in surgical procedures?
11. What surgical techniques require antimicrobial prophylaxis?
12. What environmental culturing procedures have been suggested to be most important in helping curb nosocomial disease?
13. What hygienic practice is known to reduce the incidence of nosocomial infection by at least 50 percent?

ADDITIONAL READINGS

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39. NONSPOREFORMING ANAEROBIC BACTERIA

OBJECTIVES

To list the major groups of nonsporeforming anaerobic bacteria

To outline the techniques available for isolating, cultivating, and identifying anaerobic bacteria

To outline those factors believed to be associated with pathogenesis by anaerobic bacteria

To list the anaerobic bacteria most frequently isolated from infections involving the brain, respiratory tract, digestive tract, female genital tract, soft tissue, and blood

To list the antimicrobials that are licensed for treatment of anaerobic infections

The Anaerobic Environment
Major Groups of Anaerobes
Isolation, Cultivation, and Identification
  Specimen Collection and Transport
  Cultivation
    Anaerobic Jar
    Anaerobic Glove Box
    Roll Tubes
    Bio-Bag
  Identification
Pathogenesis of Infections Caused by Nonsporeforming Anaerobes
  Brain Abscesses
  Respiratory Tract Diseases
    Upper Respiratory Tract Diseases
    Lower Respiratory Tract Diseases
  Intraabdominal Diseases
  Female Genital Tract Diseases
    Gynecological Diseases
    Obstetrical Diseases
  Soft Tissue Diseases
    Necrotic Cellulitis
    Crepitant Cellulitis
    Clostridial Myonecrosis (Gas Gangrene)
    Synergistic Necrotizing Cellulitis
  Bacteremia
Susceptibility Testing of Anaerobes
Treatment

Anaerobic nonsporeforming bacteria are normal inhabitants of humans and outnumber all other groups of bacteria indigenous to humans. Anaerobes are found on the skin, in the mouth and upper respiratory tract, in the intestinal tract, and in the female genitourinary tract. They had not been recognized as potential pathogens for many decades because of difficulties with their isolation. Much of this difficulty was due to the lack of adequate techniques and procedures for transporting and isolating anaerobes once they were removed from the infected tissue. Many of the nonsporeforming anaerobes are more sensitive to oxygen and other environmental conditions than are, for example, the clostridia. Unless specific anaerobic techniques for isolation and transport are used, these sensitive anaerobes will fail to grow. In addition wounds are likely to be contaminated with aerobes or facultative anaerobes. During isolation, even under anaerobic conditions, the facultative species often outgrow the obligate anaerobes, making isolation difficult. And when the anaerobe is isolated, one cannot always be sure that the isolated species is the causal agent of the disease.

Since anaerobes are part of the indigenous flora of humans, their role in disease is determined by alterations in the host, including surgical manipulations, trauma, antibiotic and corticosteroid chemotherapy, and underlying illness. Most nonsporeforming anaerobes do not produce identifiable virulence factors such as toxins, and their pathogenic potential is not readily apparent. The fact that most anaerobic infections in humans are mixed has led many workers to believe that bacterial synergism plays the predominant role in these diseases. For example, necrosis of mucous membranes has been observed to require the presence of four different types of organisms—three anaerobes of the genus *Bacteroides* and one facultative diphtheroid. The diphtheroid apparently supplies vitamin K, which is required for the growth of one of the bacteroides. Further, the production of acid by the diphtheroid reduces the oxidation-reduction potential of the tissue, thereby supporting the growth of the anaerobes.

Recent technological advances have increased the rate of recovery of anaerobes from infected tissue. Anaerobes are now recognized as having an important part in a wide variety of clinical disorders. A greater understanding of their characteristics is part of the education of the clinician as well as other allied health personnel.

THE ANAEROBIC ENVIRONMENT

We discussed in earlier chapters (see Chapters 6 and 8) the sensitivity of anaerobes to oxygen and the role played by superoxide dismutase. Some anaerobes produce superoxide dismutase yet are sensitive to oxygen. Apparently other factors affect the anaerobe's ability to withstand various concentrations of oxygen.

Some workers believe that the oxidation-reduction (redox) potential (Eh) of the medium is the primary factor influencing the growth of anaerobes. Bacteria, both aerobes and anaerobes, differ in their ability to grow in fresh media, depending on the initial Eh of the medium. The Eh is actually a measure of the reduced state of the medium or the capacity of the medium to contain excess electrons. Anaerobes require the greatest reducing conditions for the initiation of growth. Once growth begins, the medium will become more reduced, depending on the substrates metabolized. In an aerobic environment containing aerobic and anaerobic microorganisms the aerobic bacteria ferment carbohydrates, resulting in the production of acids that help reduce the medium and allow the growth of anaerobes. The Eh of a solution is expressed in volts or millivolts (mv). The standard used in redox potential measurements is the hydrogen half-cell at pH 0. At pH 0 the Eh of the hydrogen electrode is 0 mv, but at pH 7 it is −420 mv. Thus as the pH increases by one unit, the Eh becomes 60 mv more negative. The Eh of the standard oxygen electrode at pH 7 is +810 mv. Oxygen concentration is also related to Eh, as is pH. Increasing the oxygen tension increases the Eh to more positive values and prevents the growth of anaerobes. The redox potential of the medium can be measured electrically or by using certain dyes in the medium that have known redox potential. As the medium becomes reduced, the dyes turn colorless, and this reaction indicates the relative Eh.

Reducing conditions can be established in media by the addition of certain reducing agents such as thioglycolate, cysteine, sodium sulfite, metallic iron, and dithiothreitol. Reducing agents in the medium, in addition to absorbing oxygen, may reduce any hydrogen peroxide present and thus permit the growth of some anaerobes in the presence of oxygen.

In the host, when the Eh of tissue falls below certain levels, anaerobes can proliferate and sometimes invade tissue. Normal healthy tissue has an Eh of +150 mv. When the Eh falls below this value, anaerobes can grow. The following conditions may lead to reduced Eh values in tissue: (1) loss of vascular supply to tissue because of trauma, foreign bodies, or pressure from casts; (2) acid production by aerobes or facultative anaerobes; and (3) tissue necrosis from trauma, infection, or surgical manipulation.

MAJOR GROUPS OF ANAEROBES

The nonsporeforming anaerobes include representatives of both gram-positive and gram-negative cocci and bacilli, as well as spirochetes. The sporeforming clostridia are discussed in Chapter 21, the spirochetes in Chapter 28, and the actinomycetes in Chapter 31. Table 39-1 outlines the major anaerobic nonsporefoming genera (or species) that are pathogenic to humans.

ISOLATION, CULTIVATION, AND IDENTIFICATION

SPECIMEN COLLECTION AND TRANSPORT

Anaerobes involved in infection are generally found in areas of the body that also contain large numbers of contaminating aerobic and facultatively anaerobic species. The proper collection of specimens for analysis is crucial to the final isolation of anaerobic species. The clinical specimen should be aspirated with needle and syringe whenever feasible. Swabs should be used only if aspirates cannot be taken. Unacceptable laboratory specimens for

Table 39-1. Principal Nonsporeforming Anaerobes Implicated in Human Disease

Morphology	Genus or species	Normal flora location
Gram-positive rods	*Propionibacterium*	Skin
	Arachnia	Oral cavity, female genital tract
	Bifidobacterium	Oral cavity, intestinal tract, vagina
	Lactobacillus	Oral cavity, intestinal tract, vagina
	Actinomyces (see Chapter 31)	Oral cavity
	Eubacterium	Oral cavity, intestinal tract
Gram-negative rods	*Fusobacterium*	Oral cavity, vagina, intestinal tract
	Bacteroides	
	B. fragilis	Intestinal tract, vagina
	B. oralis	Oral cavity
	B. gingivalis	Oral cavity, vagina
	Treponema	Oral cavity
Gram-positive cocci	*Streptococcus*	Oral cavity, vagina, intestinal tract
	Peptostreptococcus	Oral cavity, intestinal tract
	Peptococcus	Oral cavity, vagina, intestinal tract
Gram-negative cocci	*Veillonella*	Oral cavity, vagina, intestinal tract
	Acidaminococcus	Intestinal tract

collection are throat swabs, saliva, nasotracheal aspirates, vaginal or cervical secretions, feces, and superficial wound swabs. Such specimens are routinely contaminated with indigenous microorganisms and are not likely to yield positive cultures of the anaerobic infectious agent. The contents of abscesses and specimens obtained from fluids and areas of the body that are normally sterile (for example, bile, blood, joints, pleural cavity, pericardial area) are likely sources of positive cultures. Some special techniques for specimen collection are percutaneous transtracheal aspiration or direct lung puncture for chest infections, aspiration of fluid from rectouterine excavation by puncture in the vaginal wall (culdocentesis) for infections of the female genital tract, and suprapubic bladder aspiration for genitourinary infections.

Once the specimen has been collected in the syringe, it should be injected immediately into an anaerobic transport vial that has been flushed with oxygen-free gases. The vial should also contain an Eh indicator such as resazurin as well as cysteine. Cysteine maintains reduced conditions if air is accidentally trapped in the vial. Specimens can also be transported by the minijar method. In this technique a 35-mm film container is partially filled with steel wool that was previously immersed in a 5% acidified solution of copper sulfate. The tissue specimen is injected into a 1-dram vial that is placed in the film container, which is then capped. The steel wool will absorb excess oxygen from the container. The clinical specimen should be inoculated as soon as possible into suitable media.

CULTIVATION

The media used for isolation of anaerobes are generally complex. Liquid culture media should never be used as the sole method of isolating anaerobes. Recovery is best obtained on selected agar media. The following media should be used for specimen processing:

1. Enriched blood agar. Enrichments include hemin, vitamin K, and animal blood.
2. Kanamycin–vancomycin–laked blood agar. This medium inhibits growth of most facultative organisms and allows growth of species of *Bacteroides*.
3. *Bacteroides* bile esculin agar. This medium is used for rapid detection of members of the *Bacteroides fragilis* group.

4. Phenylethyl alcohol blood agar. This medium supports growth of most anaerobes.

A nonselective liquid medium such as supplemented thioglycolate can be inoculated with the clinical specimen as a backup for the plate cultures. Other liquid media supplemented for isolation of anaerobes include prereduced, anaerobically sterilized chopped meat–glucose broth, and brain-heart infusion–supplemented broth.

Growth of extremely sensitive anaerobes requires the use of special techniques and procedures; which are discussed in the following paragraphs.

Anaerobic Jar

Anaerobic jars such as the GasPak are best suited for isolation of anaerobes that are only moderately sensitive to oxygen. Very oxygen-sensitive microorganisms can also be isolated using the GasPak provided certain precautions are taken. As was described in Chapter 7, anaerobiosis is established in the jar by inserting the GasPak envelope, which evolves hydrogen and carbon dioxide when water is added. The catalyst in the jar (palladium-coated alumina pellets) can become inactivated by moisture and should be replaced frequently or reactivated by heating to 170°C for 2 hours. In some anaerobic jars air can be removed by means of a pump, and the jar can later be filled with a mixture of gases containing 10% hydrogen and 5 to 10% carbon dioxide plus nitrogen. Plates to be used in the jar should be prepared and stored in an oxygen-free atmosphere. An indicator of anaerobiosis such as a filter strip saturated with dye should also be present in the jar to indicate atmospheric failures.

A disadvantage of the GasPak system relates to the inspection of cultures. Most cultures are inspected daily in the microbiology laboratory. Since the plates are stacked in the jar, it is necessary to open the jar for observations. A brief exposure to air could be detrimental to very sensitive anaerobes.

Anaerobic Glove Box

The anaerobic glove box is made of a flexible vinyl plastic with openings for neoprene gloves (Fig. 39-1). The box may enclose up to 30 cubic feet. A mixture of nitrogen, hydrogen, and carbon dioxide fills the box and maintains its shape. Agar media are prepared outside the box and placed in the box until they become reduced. Specimens are then inoculated onto the prepared agar plates within the box. An incubator may be placed in the box for incubation of the culture. This is an expensive method requiring considerable space and numerous materials.

Roll Tubes

The original roll tube technique has been modified by the use of bottles instead of tubes to provide a flat surface for inoculating specimens. Prereduced media are dispersed anaerobically into medicine bottles, which are closed with rubber stoppers and autoclaved. Platinum or stainless steel (but not Nichrome) inoculating loops are used to inoculate the media. During inoculation a flow of oxygen-free gas prevents the entrance of air. The stopper is replaced and the bottle incubated. The roll tube method has the advantage that bacteria are never exposed to air and cultures can be examined daily without interrupting the anaerobic environment. The major disadvantages are expense and length of time required to prepare media.

Bio-Bag

The Bio-Bag (Fig. 39-2) consists of a clear, gas-impermeable bag, an ampule containing an indicator, and a gas generator ampule. One or two plates are placed inside the bag, and then the bag is sealed. The indicator ampule is crushed, and the gas generator is activated. Reducing conditions can be generated in one-half hour. The plates are easily observed, and the organisms grow and remain viable up to 1 week.

IDENTIFICATION

Plates should be incubated a minimum of 48 hours before examination for growth. Those showing no growth should be reincubated for 3–5 days be-

Fig. 39-1. The anaerobic glove box. (Courtesy Coy Laboratory Products, Inc., Ann Arbor, MI.)

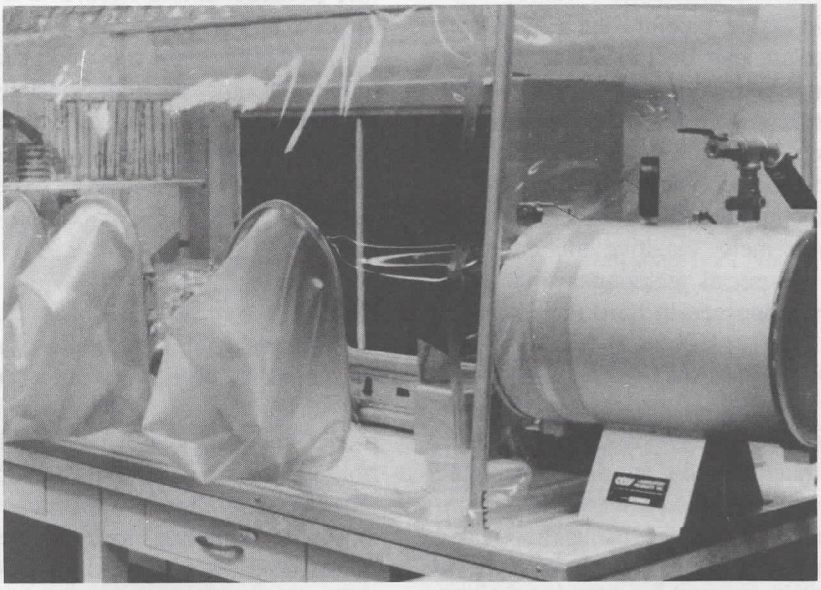
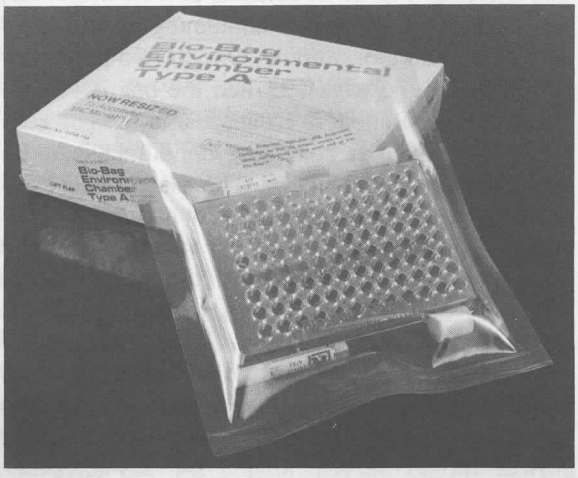


Fig. 39-2. Bio-Bag environmental chamber (type A), a disposable environmental system for the isolation of anaerobic bacteria. The chamber is sized to hold either a standard Petri dish, a minimal inhibitory concentration plate, or the miniature identification systems currently used in clinical laboratories. (Courtesy Marion Laboratories, Inc., Kansas City, MO.)



fore being discarded. Growth on anaerobic plates should be compared with growth on aerobic plates since facultative anaerobes will grow on both plates. Subcultures should be made only of colonies that have Gram-staining and colonial morphology characteristic of anaerobes. If time and facilities permit, the subcultures should be incubated anaerobically at differing concentrations of oxygen to determine the organism's tolerance to oxygen. Once the organism has been demonstrated to be an obligate anaerobe, further tests may be used to identify the species.

Identification of isolates in most laboratories is based on preliminary grouping tests and supplemental biochemical reactions. Preliminary grouping procedures include observing and recording colony morphology, pigmentation, hemolysis, pitting of agar, fluorescence, catalase production, nitrate reductase activity, and antibiotic susceptibility. Laboratories maintain anaerobic isolates in pure culture to be sent to a reference laboratory that performs definitive tests. Most definitive identification requires the use of gas-liquid chromatog-

raphy (GLC) for end product analyses. For example, the gram-positive nonsporeforming bacilli are identified by their fatty acid end products using GLC. Definitive identification is useful for determining which anaerobes are most frequently isolated from a particular type of disease. In addition definitive identification can be important in case of recurrent illness, that is, one can determine whether a new species is the cause of the disease or the original agent is still involved.

PATHOGENESIS OF INFECTIONS CAUSED BY NONSPOREFORMING ANAEROBES

More than 300 anaerobic species reside in or on the human body, but only a few of these are consistently isolated from serious disease. This implies that these few species possess special traits or determinants that enable them to cause disease. The most widely studied of the nonsporeforming anaerobes is *Bacteroides fragilis*. This organism is a frequent isolate particularly from intraabdominal disease and associated bacteremia. Some of the virulence determinants of *B. fragilis* and other nonsporeforming anaerobes will now be described.

A reduced environment may be the single most important virulence factor for anaerobes. Anaerobes that are extremely sensitive to oxygen are seldom involved in anaerobe-caused diseases. Most of the anaerobes found at the site of disease produce the enzymes superoxide dismutase and catalase. Thus low levels of oxygen may be adequately handled by these enzymes until the environment at the site of infection becomes more anaerobic and suitable for growth of pathogenic anaerobes.

Several enzymes produced by nonsporeforming anaerobic bacteria, particularly *Bacteroides* and *Fusobacterium* species, may be determinants of pathogenicity. (The enzymes produced by *Clostridium* are discussed in Chapter 21.) These enzymes include heparinase, collagenase, hyaluronidase, glycosidases, lipases, and nucleases. Together these enzymes probably contribute to abscess formation, but their individual roles in pathogenicity are not known. Heparinase produced by *Bacteroides fragilis* is believed to contribute to intravascular clotting, which leads to a requirement for increased dosages of heparin in the treatment of such conditions. The collagenase produced by *Bacteroides melaninogenicus* is believed to be associated with periodontal disease (see Chapter 37). Beta-lactamase produced by *B. fragilis* makes the organism resistant to penicillins and cephalosporins. *Bacteroides* species also produce enzymes such as DNase, RNase, and phosphatase that may facilitate invasion of tissue by liquefying exudates encountered at the site of infection.

Two of the virulence factors attributed to *Fusobacterium necrophorum* are *leukocidin* and *hemolysin*. In vivo these toxins inhibit the emigration of leukocytes to the site of infection and lyse leukocytes at the margin of the infection. This activity protects the microbial pathogens from phagocytosis. Mutants of *F. necrophorum* lacking these toxins are not as infective as strains that produce them.

Encapsulation is a virulence determinant for *Bacteroides gingivalis* and strains of *B. fragilis*. Encapsulated strains are more resistant to phagocytosis and killing by neutrophils than are nonencapsulated strains. In animal models it has been demonstrated that challenge with the purified capsular polysaccharide of *B. fragilis* induces the formation of abscesses. Animals immunized with capsular polysaccharide are protected from *B. fragilis* infection even though abscess formation can be induced by other anaerobes. Studies have also indicated that the capsular polysaccharide influences attachment to peritoneal mesothelium. Adherence to the mesothelium, for example, is blocked if the tissue is first treated with purified *B. fragilis* capsular polysaccharide.

Some anaerobes possess adhesins that contribute to virulence. Pilus-like structures have been identified in species of *Bacteroides* and *Fusobacterium*. Black-pigmented *Bacteroides* species, for example, have been shown to adhere to red blood cells, gram-positive bacteria, and epithelial surfaces. Lipopolysaccharide isolated from *Bacter-*

oides exhibits a number of biological activities in vitro that are usually not associated with the endotoxin derived from *Salmonella* species. The lack of endotoxic activity for *Bacteroides* in animal models may also explain the absence of toxic shock when this organism is the causal agent in human disease.

Anaerobes are implicated in brain abscesses, respiratory tract diseases, intraabdominal diseases, diseases of the female genital tract and the soft tissues, and bacteremia.

BRAIN ABSCESSES

Anaerobes have been isolated from brain abscesses at a very high rate. Diseases of the brain may be the result of neurosurgery, metastatic dissemination from other infected sites such as the middle ear, or extensions from areas of chronic suppurative diseases such as lung abscesses. The most frequently recovered isolates are members of the genera *Peptostreptococcus, Bacteroides, Fusobacterium*, and *Actinomyces*.

RESPIRATORY TRACT DISEASES

Upper Respiratory Tract Diseases

Most common of the upper respiratory tract diseases caused by anaerobes are periodontitis, chronic sinusitis, otitis media, and mastoiditis. Species of *Fusobacterium, Peptostreptococcus*, and *Bacteroides* are the principal microorganisms encountered in these diseases. *B. gingivalis* is found in large numbers in diseases involving the gingiva or periodontal tissue. It produces the enzyme collagenase, which no other oral microorganism does. Since collagen is the major supportive component in tissue, the enzyme is believed to be a pathogenic factor for *B. gingivalis* in oral diseases.

Lower Respiratory Tract Diseases

Lower respiratory tract diseases caused by anaerobes take two major forms: aspiration pneumonitis and lung abscess.

Aspiration pneumonitis is a mixed disease resulting from aspiration of material from the oral cavity. If the patient has poor oral hygiene and suffers from gingivitis or periodontitis, anaerobic species may be aspirated into the lower respiratory tract. Some conditions that promote the flow of upper respiratory secretions to the lower respiratory tract are alcoholism, general anesthesia, narcotic addiction, tonsillectomy, and tooth extraction. *Peptococcus* and *Peptostreptococcus* species are the major anaerobes isolated from this type of disease.

Lung abscesses may be solitary (primary lung abscesses) or multiple (necrotizing). Most infections are mixed and also result from the aspiration of oral material. The major anaerobic species isolated are *Fusobacterium nucleatum, B. gingivalis, B. fragilis*, and species of *Peptococcus* and *Peptostreptococcus*.

INTRAABDOMINAL DISEASES

Diseases of the abdomen usually stem from contamination by the contents of the gastrointestinal tract. Such contamination can be caused by gunshot wounds, stab wounds, surgery, cancer, or inflammatory bowel disease. Initially there is peritonitis, with predominantly aerobic microorganisms such as *E. coli* and species of *Klebsiella* and *Proteus*. If the peritonitis progresses into the formation of localized abscesses, anaerobic species predominate. The anaerobes usually isolated are *B. fragilis* and species of *Peptococcus* and *Peptostreptococcus*.

FEMALE GENITAL TRACT DISEASES

Female genital tract diseases can be divided into gynecological and obstetrical infections.

Gynecological Diseases

Vulvovaginal pyogenic diseases are caused primarily by anaerobic streptococci and seldom by aerobic microorganisms.

Endometritis (inflammation of endometrium of

the uterus) and pyometra (collection of pus in uterus) occur mainly in postmenopausal women, especially those with cancer of the cervix. Anaerobic streptococci and bacteroides are found as the exclusive isolates in as many as 40 percent of the cases.

Acute salpingitis and pelvic peritonitis result from bacterial invasion of the oviduct that eventually spreads to structures in the uterine cavity. Aspirates (obtained by culdocentesis) have revealed anaerobic *Peptococcus* and *Peptostreptococcus* organisms as the predominant anaerobes isolated. In these diseases *Neisseria gonorrhoeae* is the organism ordinarily isolated from the cervix, leading to the belief that the presence of *N. gonorrhoeae* predisposes the cervix to the invasion by the anaerobes.

Tuboovarian abscesses result from pelvic inflammatory disease. Culdocentesis has demonstrated that species of *Bacteroides* are the principal anaerobes present.

Suppurative pelvic thrombophlebitis originates from the uterine sinusoids. Emboli are formed there and disseminate to the lung, liver, spleen, or kidney, causing the formation of abscesses. The major clinical symptoms are chills and fever. Bacteroides and gram-positive anaerobic streptococci have been associated with this disease. The bacteroides elaborate enzymes that break down heparin, and many investigators believe that this is the major pathogenic factor in the disease.

Obstetrical Diseases

Amnionitis occurs during pregnancy, usually as a result of premature rupture of the membranes. The contaminated amniotic fluid is foul smelling and can be a cause of bacteremia in the newborn. The prognosis for the fetus is poor when bacteremia exists.

Endometritis generally occurs during the first 3 days after childbirth. Symptoms include fever and vaginal discharge (lochia) that is foul smelling. Bacteroides and gram-positive anaerobic streptococci are the principal microbial species isolated.

Septic abortion is a disease that often leads to bacteremia. Bacteroides, fusobacteria, and gram-positive anaerobic streptococci are not only the primary anaerobes isolated but represent the major groups of microorganisms isolated. A rare form of postabortal sepsis, and the most feared, is clostridial necrosis (gas gangrene) of the myometrium with accompanying toxemia and disseminated intravascular coagulation. Bacteremia caused by *Clostridium perfringens* with no systemic toxicity is not unusual in patients with other underlying illnesses. Results of bacteriological examination and identification of clostridia should be compared with clinical symptoms before treatment is initiated, especially treatment involving surgical procedures.

SOFT TISSUE DISEASES

Soft tissue diseases are often due to injury of tissue by surgery, trauma, or ischemia (deficient blood supply). The majority of infections show no tissue inflammation and are associated with aerobic and anaerobic isolates. Patients most likely to be affected are those compromised by diabetes or vascular insufficiency or both. The most common and severe syndromes are the following.

Necrotic Cellulitis

Necrotizing cellulitis is characterized by extensive necrosis of superficial fascia and systemic toxicity. Most cases occur after minor abdominal surgery. Mortality may run as high as 30 percent. The predominant anaerobes associated with this disease are *Peptostreptococcus, Bacteroides*, and *Fusobacterium* species.

Crepitant Cellulitis

Crepitant cellulitis is characterized by gas in connective tissue and minor systemic toxicity. There is no involvement of muscle. Bacteroides, peptostreptococci, and clostridia are the major anaerobic groups found in clinical isolates.

Clostridial Myonecrosis (Gas Gangrene)

See Chapter 21 for a discussion of clostridial myonecrosis.

Synergistic Necrotizing Cellulitis

Synergistic necrotizing cellulitis is similar to gas gangrene in that deep tissue is involved and there is severe systemic toxicity. The infection is located primarily in the perineal area (area in front of the anus) and in the lower extremities. Many organisms are recovered from clinical isolates, including aerobic gram-negative bacilli and anaerobic gram-positive streptococci and bacteroides.

BACTEREMIA

Anaerobes may be responsible for as much as 10 percent of all bacteremias encountered in the hospital. The predominant group isolated is the gram-negative bacilli, with *B. fragilis* accounting for more that 50 percent of the anaerobes recovered. The anaerobic gram-positive streptococci and clostridia may be found in up to 3 percent of all cases of bacteremia. Most anaerobic infections arise by displacement of microorganisms from the abdominal and pelvic tract sites; most aerobic infections, by displacement of microorganisms from the urinary tract. The major complications from bacteremia are jaundice, thrombophlebitis, and septic shock.

SUSCEPTIBILITY TESTING OF ANAEROBES

The routine testing of anaerobes for their susceptibility to antimicrobials is not recommended for most anaerobic isolates. Susceptibility testing should be reserved for cases of bacteremia, septic arthritis, osteomyelitis, and cerebral abscess. There are several reasons for this decision. The techniques required for testing anaerobe susceptibility are not the conventional techniques. Anaerobic diseases are usually polymicrobial, and thus susceptibility tests would have to be run on multiple isolates. Finally, several days are required for test results, and therapeutic decisions must be made before such results are available. Despite these problems susceptibility tests should be performed if for no other reason than to monitor sensitivity patterns of isolated anaerobes.

Several of the anaerobes have now shown resistance to penicillin. Some laboratories have reported plasmid-transferable resistance to the drugs clindamycin and erythromycin in anaerobes. Thus continual surveillance employing susceptibility tests will be required to detect the new wave of antimicrobial-resistant species.

As with aerobic and facultative anaerobes, the disk diffusion test is the most convenient technique for the diagnostic laboratory. Colonies of the strain to be tested are inoculated into thioglycolate medium enriched with hemin and vitamin K_1. After suitable growth has been obtained, in 48–72 hours, an adjusted inoculum is swabbed on freshly prepared blood agar plates containing vitamin K_1. Antibiotic disks are applied to the blood agar surface, and after 24 hours' incubation at 37°C the zones are read. Interpretation of these tests is dependent on adherence to certain standards. The susceptibility of anaerobes to several of the more commonly prescribed antibiotics is presented in Table 39-2.

TREATMENT

Antimicrobial therapy should be initiated promptly and should be such that it covers all anaerobes that may conceivably be involved in the infection. In most anaerobic infections treatment with antimicrobials follows surgical drainage or debridement. Lung abscesses, empyemas (pus in various body cavities), female genital tract infections, and crepitant cellulitis require surgical drainage before antimicrobial therapy is instituted. Tissue debridement is often necessary in synergistic necrotizing cellulitis, crepitant cellulitis, and clostridial myonecrosis (gas gangrene). Once these surgical procedures have been done, the spread of the infection into adjacent healthy tissue can be checked by antimicrobial therapy.

The antibiotics approved by the Federal Drug Administration for the treatment of anaerobic

Table 39-2. Antimicrobial Drugs Most Active Against Anaerobic Bacteria

Drug	Aerobic spectrum	Toxicity	Penetration of cerebrospinal fluid	Effect on normal flora	Dosage form available		Bactericidal activity
					Oral	Parenteral	
Penicillin	Poor	Low	Good	Minimal	Yes[a]	Yes	Very good
Clindamycin	Poor	Low–moderate	Poor	Major	Yes	Yes	Moderate
Metronidazole	None	Low	Excellent	Minimal	Yes	Yes	Excellent
Chloramphenicol	Good	High	Excellent	Minimal	Yes	Yes	None
Cefoxitin	Good–very good	Low	Moderate	Moderate–major	No	Yes	Very good
Carboxypenicillins[b]	Good	Relatively low	Good	Minimal?	No[c]	Yes	Very good
Ureido penicillins[d]	Very good	Relatively low	Good	Minimal?	No[c]	Yes	Very good

[a]Parenteral form usually preferred for anaerobic infections because it facilitates higher dosage and higher blood levels.
[b]Carbenicillin and ticarcillin.
[c]Poorly absorbed by the oral route and therefore not suitable for therapy for systemic infection.
[d]Piperacillin, mezlocillin, and azlocillin.
Source: From S. M. Finegold, Antimicrobial Therapy of Anaerobic Infections. In J. Z. Yetiv and J. R. Bianchine (Eds.), *Recent Advances in Clinical Therapeutics*. New York: Grune & Stratton, 1983. Vol. 3, p. 39.

infections are penicillin, tetracycline, clindamycin, chloramphenicol, metronidazole, erythromycin, carbenicillin, and some cephalosporins (cefoxitin, cephalothin, cefazolin, and cefamandole). Therapy for anaerobic infections should be influenced by bacteriological results as well as the state of health of the patient. In persons who are seriously ill, chloramphenicol is the drug of choice until a definitive identification of the organism or organisms can be made. In moderately ill patients, penicillin G is recommended for infections above the diaphragm and clindamycin for those below the diaphragm. Penicillin is effective against most anaerobes except *B. fragilis*. For patients sensitive to penicillin, clindamycin or erythromycin is a suitable alternative. Clindamycin and vancomycin are unsuitable for treatment of brain abscesses because of their inability to penetrate the blood-brain barrier. If the infection is mixed, as intraabdominal infections are, it is best to employ a combination of antibiotics to cover both aerobic and anaerobic microorganisms. For example, gentamicin or kanamycin is used in combination with clindamycin for intraabdominal infections. Gentamicin and kanamycin are particularly effective against aerobic gram-negative bacilli. Although tetracycline is a broad-spectrum antibiotic, it is seldom used for anaerobic infections because of the number of resistant anaerobic species that have recently been isolated from clinical specimens.

Both chloramphenicol and clindamycin are toxic antibiotics; prolonged treatment with either drug could be detrimental to the patient. Clindamycin has been shown to be a cause of pseudomembranous colitis. It should be used only when it is clearly the preferred drug. A recently approved cephalosporin is moxalactam, which is being used for infections involving *Bacteroides fragilis*.

Other treatment measures may include hyperbaric oxygen treatment, polyvalent antitoxin administration for clostridial gas gangrene, and heparin administration for septic thrombophlebitis.

SUMMARY

Nonsporeforming anaerobes indigenous to the human body are now known to be important as primary agents of disease and also to be involved in diseases in which more than one microbial agent is involved. Many species are unusually sensitive to oxygen. Several techniques are available for isolating and cultivating them. Most specimens are recovered from the patient by aspiration using a syringe. Clinical specimens are collected in containers devoid of oxygen and in which reduced conditions are maintained. Among the isolation techniques are the anaerobic jar, the anaerobic glove box, roll tubes, and the Bio-Bag. The medium used to cultivate anaerobes is also specially designed and supplemented. Some of the media are specifically designed to isolate the most important disease-producing anaerobes, *Bacteroides* species. Identification of anaerobes is based on grouping tests and biochemical reactions. Species identification often requires gas-liquid chromatography and identification of acid products of metabolism.

The type of anaerobes isolated from various sites of disease include

1. Brain abscess—*Peptostreptococcus, Bacteroides*
2. Upper respiratory tract—*Fusobacterium, Peptostreptococcus, Bacteroides*
3. Lower respiratory tract—*Peptostreptococcus, Peptococcus*
4. Intraabdominal—*Bacteroides, Peptococcus, Peptostreptococcus*
5. Female genital tract—*Peptococcus, Peptostreptococcus, Bacteroides, Fusobacterium*
6. Soft tissue—*Peptostreptococcus, Bacteroides, Fusobacterium*
7. Bacteremia—*Bacteroides fragilis, Peptococcus, Peptostreptococcus*

Several enzymes produced by nonsporeforming anaerobes appear to contribute to abscess formation, but their role in pathogenesis is not known. A capsule is the major virulence determinant for *B. fragilis*.

Antimicrobial susceptibility testing is used only for very serious diseases such as brain abscesses and osteomyelitis. Susceptibility testing, however, is important for the general monitoring of sensitivity patterns of anaerobes for epidemiological studies.

The drugs used for treatment of anaerobic infections include penicillin, chloramphenicol, tetracyclines, clindamycin, metronidazole, erythromycin, carbenicillin, and some cephalosporins. For serious disease chloramphenicol is the drug of choice until a definitive diagnosis can be made.

QUESTIONS FOR STUDY

1. Which nonsporeforming anaerobes are the most frequently associated with disease?
2. What techniques must be employed for specimen collection of nonsporeforming anaerobes?
3. What are the advantages and disadvantages of the various methods of cultivating nonsporeforming anaerobes?
4. List what you consider to be the genera of nonsporeforming anaerobes most frequently associated with disease from all body sites.
5. Why is antibiotic susceptibility testing not usually recommended for anaerobic diseases? Under what conditions should susceptibility testing be attempted?
6. What is the major virulence factor of *Bacteroides fragilis*?
7. What extracellular enzymes are produced by nonsporeforming anaerobes? What role, if any, do they have in disease?

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GLOSSARY

ABATTOIR slaughterhouse

ABSCESS a localized collection of pus

ACCESSORY CELLS reticuloendothelial cells (e.g., dendritic cells and macrophages) that interact with lymphocytes in the formation of antibodies and in other immune reactions

ACQUIRED PELLICLE the thin, structureless film of salivary glycoproteins that forms on the tooth surface

ACTIVE IMMUNITY the acquired immune status that develops as a consequence of an infection or of immunization with vaccines

ACUTE having a short and relatively severe course

ADCC antibody-determined cell-mediated cytotoxicity; the destruction of IgG-coated cells by K cells

ADHESINS components on the microbial surface that are used for attachment to the host

ADJUVANT a substance used to prolong an antigenic stimulus

AEROBIC requires oxygen for growth

AEROSOL a solution delivered as a fine mist

AGGLUTINATION a reaction in which cells are clumped, as occurs in the interaction of a particulate antigen with antibody

AGGLUTININ antibody that can cause agglutination

AIDS acquired immune deficiency syndrome, an oftentimes fatal disease that especially strikes multicontact male homosexuals; probably a defect in cell-mediated immunity in which there is a preponderance of T suppressor cells over T helper cells; nonpathogens or Kaposi's sarcoma or both often cause the fatality

ALLERGY a hypersensitivity to animate or inanimate substances

ALLOGRAFT (HOMOGRAFT) a tissue or organ graft between members of the same species who are not identical

ALLOSTERIC ENZYME an enzyme whose reactivity with another molecule such as its substrate is altered because of interaction of the enzyme with other molecules

ALOPECIA loss of hair due to disease

ALTERNATIVE (ALTERNATE) COMPLEMENT PATHWAY the complement pathway that is activated at the C3 level; does not require immune products for

activation and is therefore important for nonspecific host resistance

ALVEOLAR pertaining to the small sac-like structures in the lung

ALVEOLAR BONE the type of bone supporting the teeth

AMPHITRICHOUS having one flagellum at each pole of the cell

AMYLOPECTIN intracellular storage polysaccharide synthesized by bacteria in dental plaque

ANABOLISM metabolic process involved in the synthesis of cell material

ANAEROBIC growing only in the absence of molecular oxygen

ANAEROBIOSIS life in the absence of oxygen

ANALOGS closely similar compounds that can sometimes replace an essential metabolite

ANAMNESTIC RESPONSE a memory response in which a second injection of antigen (after an initial injection) causes a sudden increase in antibody titer

ANAPHYLATOXIN complement fragments C3a or C5a that mediate intense inflammatory reactions by causing the degranulation of mast cells

ANAPHYLAXIS the state in which an individual is hypersensitive to a given antigen

ANEURYSM a sac formed from the dilation of the walls of an artery or vein and filled with blood

ANOREXIA a condition in which the person has no appetite

ANTIBIOGRAM pattern of results obtained from tests used to determine a microorganism's susceptibility to antimicrobials

ANTIBIOTIC a chemical compound produced by microorganisms that can inhibit or kill other microorganisms

ANTICODON the triplet of nucleotides in a transfer DNA molecule that complements a specific codon on the messenger RNA

ANTIGEN a foreign substance capable of inducing an immunological response

ANTIGEN-BINDING SITE the variable region of the Fab portion of the immunoglobulin molecule that binds a specific or closely related antigenic determinant

ANTIGENIC DETERMINANTS small, discrete chemical groups on the antigen surface that combine with the antigen-binding site on an immunoglobulin molecule or on a lymphocyte receptor

ANTI-IDIOTYPE an immune product that reacts with the antigenic determinants of the antigen-binding site

ANTIMETABOLITE a molecule that bears a strong resemblance to one required in a normal physiological reaction

ANTISEPTIC a chemical compound that can be used on the surface of living tissue and that inhibits bacterial growth

ANTISERUM a serum containing specific antibodies

ANTITOXIN a specific antibody capable of neutralizing the exotoxin that stimulated its production

ANUG acute necrotizing ulcerative gingivitis

ARTHRALGIA painful swollen joints

ARTHROPOD invertebrate with jointed limbs

ASCOSPORE a sexual spore of the Ascomycetes

ASEPTIC free of living microorganisms

ASPHYXIA suffocation

ASPIRATION a process in which fluids are removed from body cavities

ASSIMILATION transformation of nutrients into biologically useful compounds or structures

ATOPIC ALLERGIES common clinical allergies in which there is a hereditary predisposition to form IgE

ATROPHY the wasting away of a tissue, organ, or limb

ATTENUATION lessening; reduction of the virulence of microorganisms as in a vaccine

AUSTRALIA ANTIGEN hepatitis B surface antigen associated with serum hepatitis (hepatitis B); originally discovered in an Australian aborigine (hence the name)

AUTOCATALYSIS a process in which the product of a reaction acts as a catalyst to accelerate the reaction

AUTOCLAVE sealed chamber used for sterilizing

AUTOIMMUNE DISEASE disease in which the host's immune system destroys its own tissue

AUTOLYSIS digestion of a cell by enzymes produced by that cell at time of death

AUTOTROPH an organism that obtains energy by the oxidation of inorganic compounds

AUXOTROPH a mutant microorganism that will grow only on minimal media supplemented with growth factors not required by the normal parent

AXENIC a culture of organisms containing but one strain; also called pure culture

BACILLUS an organism of the genus *Bacillus*; also used to designate rod-shaped bacteria

BACTEREMIA presence of bacteria in the bloodstream

BACTERICIDE an agent that kills bacteria

BACTERIOCIN antimicrobial substance produced by bacteria that kills sensitive members of related strains

BACTERIOPHAGE a bacterial virus, sometimes referred to as phage

BACTERIOSTATIC an agent that inhibits the growth of bacteria

BACTERIURIA presence of bacteria in the urine

BACTOGEN see CHEMOSTAT

B CELLS the lymphocyte population involved in the production of antibodies (humoral immunity)

BENIGN not malignant; subject to recovery

BETA OXIDATION a degradation process of lipids resulting in the release of two carbon fragments

BIOSYNTHESIS the building up of chemical compounds

BIOTRANSFORMATION a process of detoxification of drugs primarily by the liver

BIOTYPE a strain of a species differing from other strains in a biochemical property

BLASTOSPORE a spore formed by budding, as occurs in yeast cells

BLOCKING ANTIBODY an antibody that can inhibit the activity of another antibody

BOIL a localized abscess resulting from infection of a hair follicle; also called a furuncle

BOOSTER DOSE an amount of immunogen given some time after primary immunization to sustain the immune response at a high level

BRONCHIECTASIS chronic dilatation of the bronchi marked by repeated coughing spells

BROWNIAN MOVEMENT dancing motion of particles in a liquid caused by thermal agitation

BUBOES lymph nodes that have become enlarged

BUCCAL pertaining to the cheek

BUDDING in virology, release of virus through cellular membranes and acquisition of cell envelope components; asexual division in prokaryotes

BULLA a blister or vesicle filled with fluid

CACHEXIA physical wasting due to ill health and/or malnutrition

CALCULUS a mineralized form of plaque on the surface of teeth in which organic components have been replaced by inorganic ones

CAPNEIC requiring elevated concentrations of CO_2 for growth

CAPSID the protein coat of a virus

CAPSOMERE an aggregate of polypeptides forming a unit of the viral capsid

CAPSULE a slimy envelope that surrounds the cell wall of certain microorganisms

CARBUNCLE a circumscribed infection of the skin or subcutaneous tissue containing multiple draining sinuses

CARIES a disease of the calcified tissue of teeth; tooth decay

CARRIER a host that harbors infectious microorganisms and can transmit them to others but shows no disease symptoms

CASEATION a type of necrosis in which the tissue resembles an amorphous mass of cheese

CATABOLISM a metabolic process in which foodstuffs are broken down to release energy

CATALYST a substance that increases the rate of a chemical reaction without being consumed in the reaction

CATARRHAL a stage during infection in which there is a discharge from inflamed mucous membranes

CATHETER a tubular device used to withdraw fluids from or introduce them into the body cavities

CELL-MEDIATED IMMUNITY (CMI) an acquired immunity in which the T lymphocytes play a major role; responsible for resistance to infectious disease, some autoimmune diseases, and certain allergies

CELLULITIS diffuse inflammation of connective tissue

CHANCRE the primary lesion of syphilis

CHEMIOSMOSIS a process in which energy is har-

nessed to produce an electrochemical gradient across cell membranes

CHEMOPROPHYLAXIS preexposure administration of drugs to prevent disease

CHEMOSTAT an apparatus used to maintain bacterial cultures in a state of continuous division

CHEMOTAXIN a leukoattractant such as an antibody or C3a and C5a

CHEMOTAXIS phenomenon of attraction in response to chemicals released in the environment

CHEMOTHERAPY treatment of disease through the administration of drugs

CHROMATIN BODY a network of DNA fibers giving the appearance of a distinct body in the cell

CHROMOSOME a rod-shaped mass of DNA that carries hereditary characteristics

CHRONIC lasting a long period of time

CIRRHOSIS a disease of the liver

CLASSIC COMPLEMENT PATHWAY the complement pathway that is activated at the C1 level, usually by the reaction of an IgG or IgM antibody with antigen

CLONAL SELECTION the theory that for each antigenic determinant there is a specific, genetically predetermined receptor on the surface of an immunocompetent lymphocyte; the combination of the determinant with the receptor activates the formation of a clone of cells, each of which forms immune products of the same antigen-binding specificity

CLONE a group of identical organisms or cells derived from a single cell or organism

COCCOBACILLUS an oval bacterial cell resembling both coccus and rod shapes

COCCUS spherical bacterium

CODON a nucleotide triplet in messenger RNA that specifies a particular amino acid

COENZYME an organic molecule that is loosely bound to the enzyme and necessary for the activity of the enzyme

COLICIN a protein secreted by certain strains of *Escherichia coli* and lethal to other strains of the same species

COLITIS inflammation of the colon

COLONY a uniform mass of cells derived from a single cell growing on a solid surface

COMMENSALISM a symbiotic relationship between different species of organisms in which neither is harmed

COMPLEMENT a series of complex thermolabile plasma proteins

COMPLEMENT SYSTEM a system of plasma proteins that upon activation proceeds in a serial (cascade) fashion to generate biologically active fragments including opsonins, chemotaxins, anaphylatoxin, and membrane attack units

CONDYLOMATA ACUMINATA warts on the genitalia

CONDYLOMATA LATA moist syphilitic papules

CONIDIA asexual fungal spores produced on specialized mycelial branches called conidiophores

CONJUGATION the act of joining together; in bacteria a process in which genetic information is transferred from one cell to another

CONJUNCTIVITIS inflammation of the membrane that lines the eyelids and the exposed surfaces of the sclera of the eye

CONSTITUTIVE ENZYME an enzyme produced by a cell under any environmental conditions

CONVALESCENCE period of recovery

COPROANTIBODIES antibodies occurring in the intestinal tract, consisting primarily of the IgA class

CORYZA inflammation of the nasal mucosa characterized by nasal discharge and watery eyes

C-REACTIVE PROTEIN a plasma protein that appears in response to inflammation in the body

CRISTAE the inner folded membrane found in the mitochondrion

CROUP a condition of children in which there is coughing and hoarseness

CRYOSURGERY use of extreme cold to destroy tissue

CULTURE a population of microorganisms growing in a nutrient medium

CYTOCHROME a respiratory pigment involved in oxidation-reduction reactions

CYTOPATHIC characterized by pathological change in a cell

CYTOPATHIC EFFECT (CPE) morphological alteration of host cells that usually results in cell death

CYTOPHILIC ANTIBODY antibody that binds to the Fc membrane-bound receptor of macrophages

DEAMINATION removal of amino groups
DEBILITATED characterized by loss of strength or health
DEBILITATION loss of normal function
DEBRIDE remove devitalized tissue
DECARBOXYLATION removal of carboxyl groups
DEGENERATE in genetics, a code in which more than one code word specifies an amino acid
DEHYDROGENATION a process in which hydrogens are removed from a compound
DELETION in genetics, loss of genetic material
DEMENTIA mental deterioration
DEMYELINATION removal of the myelin sheath around nerve fibers
DENATURATION a process in which a macromolecule loses its configuration and often its biological activity
DERMATOPHYTE fungi that invade keratinized tissue
DESENSITIZATION the process of reducing an individual's sensitivity
DESICCATE to dry
DESQUAMATION shedding of the superficial layers of skin
DETERGENT a synthetic cleansing agent
DETERMINANT GROUP the chemical grouping on the antigen molecule that reacts specifically with the antibody molecule
DEXTRO- to the right
DIARRHEA abnormal fluidity of fecal discharge
DIMORPHIC in mycology the ability of the fungus to exist in the yeast or mold state
DIPHTHEROID resembling the diphtheria bacillus
DIPLOPIA double vision
DISEASE an abnormal condition of the body having characteristic symptoms
DISINFECTANT a substance that destroys vegetative pathogens
DTP a vaccine consisting of toxoids of diphtheria and tetanus plus a pertussis bacterial antigen
DYSURIA painful urination

ECTATIC distended
ECTOTHRIX fungal infection in which hyphae remain on external surface of the hair shaft
ECZEMA an inflammatory skin disease characterized by scales, crusts, and watery lesions
EDEMA excessive accumulation of fluid in the tissues
EFFECTOR CELL a lymphocyte that has a direct (e.g., killer T cell) or a mediated (e.g., via lymphokines) effect on an antigen (compare REGULATOR CELL)
ELISA enzyme-linked immunosorbent assay, a serological test in which enzyme-linked antibodies allow antigen-antibody reactions to become visible upon addition of a color-producing enzyme substrate
EMPYEMA pus accumulation in a body cavity
ENDEMIC continually present in a community
ENDOCARDITIS inflammation of the endocardium of the heart
ENDOCYTOSIS the phase of phagocytosis when the extracellular material is entering the phagocyte
ENDOENZYME an enzyme produced by a cell whose activity is associated with intracellular processes or an enzyme that attacks the interior of macromolecules
ENDOGENOUS produced within the cell; coming from within
ENDOMETRITIS inflammation of the endometrium of the uterus
ENDONUCLEASE an enzyme that attacks the interior of nucleic acid molecules
ENDOPLASMIC RETICULUM a protoplasmic network in eukaryotic cells consisting of a continuous double membrane system that courses throughout the cytoplasm
ENDOSPORE a spore occurring within the cell
ENDOTHRIX fungal infection resulting from hyphal invasion of the hair shaft
ENDOTOXIN a toxin derived from the cell wall of gram-negative bacteria
ENTERIC occurring in the gastrointestinal tract
ENTEROTOXIN a toxin that gives rise to gastrointestinal symptoms when ingested or formed in the intestine
ENVELOPE a host cell–derived membrane, containing virus-specific antigens, which is acquired during virus maturation
EOSINOPHIL a granular leukocyte readily stained by eosin

EPIDEMIC an outbreak of a disease affecting a large number of individuals in a community
EPIDEMIOLOGY the science that deals with the incidence, transmission, and prevention of disease
EPINEPHRINE (ADRENALIN) a drug that is a vasoconstrictor and smooth muscle relaxant
EPISOME a piece of DNA that may be an autonomous unit in the cell or may be integrated into the chromosome
EPITOPE antigenic determinant
EPIZOOTIC an outbreak of disease affecting a large number of animals
ERYSIPELAS a contagious disease of the skin resulting from infection by *Streptococcus pyogenes*
ERYTHEMATOUS having red eruptions on the skin
ERYTHROGENIC able to produce redness
ESCHAR a dry mass of necrotic (dead) tissue
ETIOLOGY study of the causation of a disease
EUKARYOTIC of a cell type in which the nuclear material is bounded by a membrane
EXANTHEM eruptive disease or fever characterized by nodular eruptions on mucous membrane
EXFOLIATION shedding of layers of the skin
EXOENZYME an enzyme secreted by the cell and associated in activity with the extracellular process
EXON in eukaryotic genetics, an mRNA sequence that codes for information
EXOTOXIN toxin produced in the cell and released into the environment
EXUDATE material (fluids, cells, etc.) that has escaped from blood vessels

FAB the fraction of the immunoglobulin molecule that contains the antigen-binding site
FACULTATIVE ANAEROBES organisms that grow best under aerobic conditions but can grow anaerobically
FAUCIAL pertaining to the upper part of the throat
FC the crystallizable fragment of the immunoglobulin molecule, composed of the constant regions of the H chains; roles include binding of complement, attachment of immunoglobulin to phagocytes and other cell types, and transplacental passage
FEBRILE pertaining to or characterized by fever
FERMENTATION an anaerobic metabolic process that uses an organic compound as the final electron acceptor
F FACTOR a fertility factor that determines the sex of a bacterium
FIBROSIS formation of fibrous tissue
FILAMENTOUS composed of long thread-like structures
FIMBRIA fringe; in microbial genetics, a small protein projection on the surface of bacteria
FISSION the act of splitting, a form of asexual reproduction
FISTULA an abnormal passageway providing organisms with communication between tissues during infection
FIXED VIRUS an attenuated variant of the virulent "street" rabies virus
FLAGELLA hair-like projections on the cell that aid in locomotion
FLATULENCE presence of air or gases in the stomach or intestine
FLOCCULATION a phenomenon in which suspended components form visible discrete particles
FLORA the resident organisms in a particular area
FLUORESCENCE the emission of light while being exposed to light of shorter wavelength than the emitted light
FOCUS in virology, an accumulation of transformed cells
FOMITE an inanimate object that may be involved in disease transmission
FRAMEWORK REGIONS the less variable regions of the antigen-binding site of the antibody molecule that flank the hypervariable subregions where antigen contact actually occurs
FULMINATING occurring very suddenly and with intensity
FUNGICIDE an agent capable of destroying fungi
FURUNCLE a boil

GAMETE sex cell
GAMMA GLOBULIN a blood protein fraction with which antibodies are associated
GENOME the complete set of hereditary factors
GENOTYPE the genetic constitution of an organism
GENUS a taxonomic category
GERMICIDE an agent that destroys pathogenic microorganisms

GERMINATION the sprouting of a spore and the formation of a vegetative cell

GERM TUBE in fungal reproduction a hyphal element arising from a germinating spore

GINGIVAE (GUMS) soft tissue surrounding the teeth

GLOBULINS a class of proteins found in the blood

GLOMERULONEPHRITIS renal disease affecting primarily glomeruli

GLOSSITIS inflammation of the tongue

GLUCAN extracellular glucose polymers (dextran, mutan)

GLUCOSYLTRANSFERASE an enzyme of dental plaque bacteria that cleaves sucrose and polymerizes glucose into glucans

GLYCOLYSIS the anaerobic process in which carbohydrates such as glucose are oxidized to pyruvic or lactic acid

GNOTOBIOTIC ANIMALS animals originally germ free, harboring one or more known microorganisms

GOLGI membranous complex found in eukaryotes that packages proteins and lipids for transfer to selected sites in the cell

GRAFT-VS.-HOST REACTION (GVHR) a reaction in which the immunocompetent cells of a graft tissue attack the tissues of the graft recipient

GRANULOMA a tumor-like mass of granulation tissue containing actively growing fibroblasts and caused by chronic inflammation

GUARNIERI'S BODIES acidophilic intracytoplasmic inclusion bodies in epidermal cells infected with smallpox virus

GUMMA granuloma found in tertiary stage of syphilis

HALOPHILIC (of bacteria) requiring high concentrations of salt to maintain the integrity of the cell

HAPTEN a partial or incomplete antigen that alone does not induce antibody formation but can react with certain antibodies

H CHAIN heavy chain, one of the pair of identical polypeptides that represent two of the four polypeptide chains of the basic immunoglobulin molecule; differences in H chain composition are the basis for classification of immunoglobulins into five classes

HELPER T CELLS (T_H) a regulator T cell that helps most B cells and other T cells to respond to antigen

HEMAGGLUTINATION clumping of red blood cells

HEMAGGLUTININ (VIRAL HEMAGGLUTININ) a nonantibody protein on the outer surface of some viruses (e.g., orthomyxoviruses) that reacts with surface determinant(s) on red cells to cause agglutination of the red cells (hemagglutination)

HEMATURIA presence of blood in the urine

HEMOLYSIN any agent that can cause the lysis of red blood cells

HETEROPHILE ANTIGEN an antigen common to more than one species such as lens protein antigen

HETEROTROPH an organism requiring organic material for energy and biosynthesis

HEXON a capsomere surrounded by six capsomeres

HISTAMINE a physiologically active substance released by certain cell types, especially mast cells, that affects capillary permeability and smooth muscle contraction

HISTOCOMPATIBLE said of cells or tissues that share or have like transplantation antigens

HISTONE a protein containing many basic amino acids and associated with the DNA of eukaryotic cells

HLA human leukocyte antigens; human transplantation antigens—first discovered on leukocytes

HOMOGRAFT see ALLOGRAFT

HUMORAL IMMUNITY antibody-mediated immunity

HYBRIDOMA a cell that results from the fusion of two cells; usually refers to a cell fused from a cancerous (myeloma) cell and an antibody-producing cell and that is a source of monoclonal antibodies

HYDROLASE an enzyme that catalyzes the hydrolysis of compounds

HYDROLYZE to split a compound by the addition of water

HYDROPHILIC attracted to water

HYDROPHOBIC repelled by water

HYPERCHROMIC highly stained

HYPERPLASIA increase in the number of cells in a tissue

HYPERTONIC a solution in which the concentration of solutes is higher outside the cell than inside the cell

HYPERVARIABLE REGIONS extremely variable regions of the variable H and L regions of immunoglobulins; the "hot spots" where antibodies bind antigenic determinants

HYPHA (PL. HYPHAE) one of the filaments that make up a fungal mycelium

HYPOSENSITIZATION a process in which an allergen is injected to reduce the allergic state

HYPOTONIC a solution in which the concentration of solute is lower outside the cell than in the cell

ICOSAHEDRAL 20 sided

ICTERUS (JAUNDICE) a condition characterized by excess of bile pigments in the blood and tissues that leads to a yellow color of the surface integuments

ID an allergic skin reaction to fungi or fungal products

IDIOTOPE a single determinant of the idiotype

IDIOTYPE the set of antigenic determinants that are part of the antigen-binding site of the immunoglobulin molecule

IMMUNOFLUORESCENCE fluorescence resulting from the conjugation of an immunoglobulin to a fluorescent dye

IMMUNOGEN an antigen or a substance that induces an immunity

IMMUNOGENICITY that property of an antigen that makes it capable of stimulating an immune response

IMMUNOGLOBULINS a class of blood proteins with which antibodies are associated

IMMUNOSUPPRESSIVE inhibiting the normal immunological response of an organism

IMMUNOTOXIN in cancer immunotherapy, a toxin (e.g., ricin A) attached to a monoclonal antibody that poisons the cell against which the antibody is specific

IMPETIGO a streptococcal infection of the skin

INCLUSION BODIES in virus infection, the highly stainable components, usually virus, found in the cytoplasm or nucleus of the infected cell

INDIGENOUS native to a particular place

INDUCER a molecule capable of stimulating the formation of compounds such as enzymes involved in cellular metabolism

INDUCIBLE ENZYME an enzyme synthesized only in response to an inducer

INDURATION a hardened area or lesion

INFANT a child usually up to 2 years

INFECTIOUS able to cause disease

INFECTIOUS DOSE (ID_{50}; $TCID_{50}$) that amount of virus required to cause a demonstrable infection in 50 percent of the inoculated animals or tissue culture cells, respectively

INFLAMMATION nonspecific response to irritants (chemical, physical, microbial, antigenic) characterized by pain, heat, redness, and swelling

INFUSION a preparation in which important components of a substance have been extracted with water

INOCULUM a substance (microorganisms, serum, etc.) introduced into the tissues or culture media

INSERTION in genetics, the addition of nucleotides or genes to the chromosome

INSERTION SEQUENCE small nucleotide sequences that are capable of movement on the DNA

INSPISSATE to dry by evaporation

INTERFERON a class of proteins produced by vertebrate cells in response to viruses, endotoxins, and certain chemicals; associated primarily with antiviral activity

INTERLEUKIN an effector of immune regulation that transmits growth and differentiation signals among leukocytes

INTRADERMAL within the skin

INTRON in eukaryotic genetics, a mRNA sequence that does not code for information

INTUBATION insertion of a tube

IN UTERO within the uterus

INVASIVE able to invade or penetrate the body

IN VITRO outside the body or performed in artificial environments

IN VIVO within the body or within a living organism

IODOPHORS disinfectants consisting of iodine combined with a carrier molecule

ISCHEMIA blood deficiency in a body part

ISOAGGLUTININS antibodies specific for antigenic

sites on red blood cells of the same species and causing their agglutination; also called alloagglutinins

ISOANTIBODY an antibody that reacts with alternate forms of an intraspecies antigen (e.g., A and B blood group antigens)

ISOANTIGEN an intraspecies antigen that appears in alternate forms

ISOMER a molecule having the same atoms or groups of atoms as another molecule, but with different arrangement

JAUNDICE see ICTERUS

J CHAIN a polypeptide chain that joins basic monomer immunoglobulin units of the same immunoglobulin class to form polymers, as in secreted IgM and secretory IgA

KAPOSI'S SARCOMA a relatively benign skin cancer that often takes a more aggressive fatal form in AIDS victims

KARYOTYPE chromosomal makeup of a cell

KERATITIS inflammation of the cornea

KERNICTERUS a condition associated with high levels of bilirubin in the blood that affects the nervous system

KOPLIK'S SPOTS small bluish spots surrounded by a reddened area in the mucous membrane of the mouth and characteristic of measles

KUPFFER'S CELLS macrophages lining the hepatic sinusoids

KURU a chronic, progressive, degenerative disorder of the central nervous system caused by a virus, found among certain natives of New Guinea

LABILE unstable or susceptible to various chemical or physical agents

LATENCY a state of inactivity

LD_{50} lethal dose 50; the dose lethal to 50 percent of the subjects

LEUKEMIA a blood disease characterized by high levels of leukocytes

LEUKOCIDIN a substance produced by some pathogenic bacteria and toxic to white blood cells

LEUKOCYTE white blood cell

LEUKOPENIA a smaller than normal number of circulating leukocytes

LEUKOTRIENES secondary or lipid mediators that arise from changes in cell membrane phospholipids (arachidonic acid) especially when antigen combines with IgE on mast cell surfaces; leukotrienes increase vascular permeability, contract smooth muscles, and attract neutrophils

LEVO- to the left

L-FORMS bacteria with deficient cell walls

LIGASE an enzyme that catalyzes the joining together of two molecules coupled with the breakdown of adenosine triphosphate to adenosine diphosphate

LIMULUS LYSATE an extract from the amoebocytes of horseshoe crabs that gels with minute quantities of endotoxin

LOCAL (TOPICAL) IMMUNITY mucous surface immunity primarily owing to secretory IgA

LOCHIA vaginal discharge that appears after childbirth

LOPHOTRICHOUS having a tuft of flagella at one end of the cell

LYMPHADENOPATHY infection of a lymph node

LYMPHOCYTE a white blood cell devoid of cytoplasmic granules, associated with immune response and chronic inflammation

LYMPHOKINES soluble products, especially of sensitized T cells, that are released upon antigen contact and that have among their varied actions the mobilization of uncommitted macrophages, neutrophils, lymphocytes, and other cells

LYMPHOMA any neoplasia (abnormal growth) associated with the lymph system

LYOPHILIZATION process of freeze-drying

LYSIN an agent (bacterial, chemical, antibody) capable of causing the destruction of cells

LYSIS dissolution (as in cell destruction)

LYSOGENY a state in which a bacteriophage genome is integrated or firmly associated with the host genome

LYSOSOME a cell organelle derived from the Golgi apparatus containing hydrolytic enzymes collectively termed acid hydrolases

LYSOZYME an enzyme that degrades peptidoglycan, a component of the cell wall

MACROPHAGE a large mononuclear cell derived from the reticuloendothelial system and having phagocytic activity

MACULA a spot; often associated with a type of rash (macular) in which the lesion is not elevated

MAJOR HISTOCOMPATIBILITY COMPLEX the genes of a specific chromosome region that code for the majority of cell membrane surface glycoproteins that serve as transplantation antigens and that guide antigen recognition and cell interactions, especially by T cells

MALAISE a general feeling of "not being well"

MEDIUM a substance that provides nutrients for the growth of microorganisms

MENINGES membranes that cover the brain and spinal cord

MENINGITIS inflammation of the membranes (meninges) of brain or spinal cord

MESOPHILE a microorganism that grows best at temperatures between 20° and 45°C

MESOSOME involuted membrane of the bacterial cell

METABOLISM the sum total of physical and chemical changes that take place in a cell and maintain the cell's integrity

METABOLITE a product of metabolic processes

METACHROMATIC GRANULES cytoplasmic inclusions that are polymetaphosphates; also called Babès-Ernst bodies or volutin

METASTASIS transfer of disease from primary site of infection

MHC major histocompatibility complex

MHC RESTRICTION the limitations imposed by the MHC on the ability of T cells to recognize antigens and to interact with other cell types

MICROAEROPHILE an organism requiring less than 1 atmosphere of oxygen

MICROTUBULE cylindrical hollow protein tubes found in eukaryotic cells and associated with motility as well as spindle organization during cell division

MIGRATION INHIBITING FACTOR (MIF) a lymphokine that prevents macrophages from leaving the site of cell-mediated immunological reaction

MILIARY resembling millet seeds; said of lesions

MISSENSE refers to a mutation that results in a codon's being translated into a different amino acid

MITOCHONDRION rod-shaped organelle found in eukaryotic cells and involved in energy production

MOLD another name for fungi that exhibit branching

MONOCLONAL ANTIBODY antibody obtained from an isolated clone of cells (e.g., hybridoma); preparations containing monoclonal antibodies have a highly precise specificity for a given antigenic determinant and are therefore valuable in diagnosis and therapy

MONOCYTE a macrophage of the circulating blood

MONOMER the simplest molecule of a compound

MONOTRICHOUS having one flagellum

MORBIDITY sickness; the ratio of sick to well individuals of a community

MORDANT a chemical that when added to a dye makes it stain more intensely

MORPHOLOGICAL related to shape or structure

MORTALITY fatality; the ratio of the number of deaths to a given population in a defined situation

MUCOLYTIC capable of dissolving mucus

MUTAGEN a substance that increases the mutation rate of an organism

MUTATION a process in which a gene undergoes structural changes

MYALGIA pain in the muscles

MYCELIUM a mat of intertwined hyphae of fungi

MYCETISMUS mushroom poisoning

MYCOTOXIN toxic secondary metabolite resulting from the growth of fungi on grains

MYOCARDITIS a condition involving the heart muscle (myocardium)

MYOMETRIUM the uterine muscular structure

MYOSITIS inflammation of muscle

NATURAL KILLER (NK) CELLS probably a subclass of lymphocytes that occur without antigen stimulation and that serve as effectors of nonspecific immunity (e.g., against malignant cells)

NECROSIS death of tissue or cells

NEGRI BODIES inclusion bodies found in brain cells infected with the rabies virus

NEMATODE roundworm

NEONATAL pertaining to the first 4 weeks after birth

NEPHROTOXIC toxic to the kidney
NEUROTOXIN toxin that affects the nervous system
NEUTROPHIL a leukocyte in which granules do not stain with basic or acid dyes
NITROGEN FIXATION the union of nitrogen gas with other elements to form chemical compounds
NK CELLS natural killer cells
NONSENSE a mutation that results in a codon's not being translated into an amino acid
NOSOCOMIAL pertaining to the hospital
NUCLEOCAPSID a unit of viral structure consisting of a protein coat (capsid) and the nucleic acid it encloses
NUCLEOID area of DNA concentration in the bacterial cell
NUCLEOLUS a small body within the nucleus rich in ribonucleic acid
NUCLEOSIDES a class of compounds consisting of a carbohydrate and a purine or pyrimidine base
NUCLEOTIDES a class of compounds consisting of a purine or pyrimidine base, phosphoric acid, and carbohydrate
NUTRIENT a substance removed from the environment by cells and used in various metabolic processes

OBLIGATE necessary or required
OCCLUSAL SURFACE area of the tooth associated with grinding
OKAZAKI FRAGMENT short pieces of DNA synthesized during replication
OLIGURIA secretion of a diminished amount of urine
ONCOGENIC able to cause tumors
ONYCHIA infection involving the nails
OPERATOR GENE a chromosomal region that is capable of controlling adjacent structural genes
OPERON a unit on the chromosome consisting of adjacent genes controlled by an operator
OPPORTUNISTIC PATHOGEN a nonpathogen capable of infection only under the most favorable conditions
OPSONIN an antibody or complement fragment C3b that attach antigens to phagocytes, thereby facilitating endocytosis
ORCHITIS inflammation of the testes
ORNITHOSIS a bird disease, transmissible to humans
OSMOSIS passage of fluids through a semipermeable membrane
OSTEITIS inflammation of a bone
OSTEOCLAST a bone-resorbing cell
OSTEOMYELITIS inflammation of the marrow of the bone
OTITIS MEDIA inflammation of the middle ear
OXIDATION loss of electrons by a compound
OXIDATIVE PHOSPHORYLATION enzymatic addition of phosphate to adenosine diphosphate to form adenosine triphosphate, which is coupled to the electron transport system
OZENA chronic inflammation of the nose

PALINDROMIC in genetics, nucleotide sequences that are repeated but inverted on the opposite ends of a double-stranded nucleic acid molecule
PANDEMIC a worldwide epidemic
PAPILLOMA a benign tumor derived from the epithelium
PAPULE a small, firm, circumscribed, raised lesion of the skin
PARASITE an organism that survives on or at the expense of a living host
PARONYCHIA infection involving areas around nails
PAROXYSM a state of intensified symptoms
PARTURITION the process of birth
PASSIVE IMMUNITY the temporary acquired immune status that results from the transfer of antibodies from an outside source
PASTEURIZATION a process in which fluids are heated at temperatures below boiling to kill pathogenic microorganisms in the vegetative state
PATHOGEN an organism capable of habitually causing disease in a percentage of healthy persons
PATHOGENESIS the progression of tissue, biochemical, and functional alterations that occur during the development of a disease
PATHOGNOMONIC refers to a sign or symptom that is so characteristic of a disease that a diagnosis can be made from it
PENTON a capsomere surrounded by five capsomeres

PEPLOMER a projection (spike) extending from the outer surface of a virus envelope

PEPTIDE BOND a bond that unites two amino acids

PERCUTANEOUS performed through the skin

PERINATAL pertaining to a period of time shortly before and just after birth

PERIODONTITIS inflammation of the periodontium or tissue surrounding and supporting the teeth

PERIOSTITIS inflammation of the periosteum, the specialized connective tissue covering all bones

PERITRICHOUS having flagella that cover the entire bacterial surface

PERMEASE an enzyme found in cell membranes that transports compounds into the cell cytoplasm

PETECHIA a pinpoint hemorrhagic spot

PHAGOCYTOSIS the process of ingestion of foreign particles

PHAGOSOME a cell vacuole resulting from phagocytosis of particulate materials

PHENOTYPE the genetic makeup of an organism; its observable properties

PHLEBITIS inflammation of a vein

PHOSPHORYLATION joining of phosphoric acid to a compound

PHOTOPHOBIA literally, fear of light; painful sensitivity to light

PHOTOSYNTHETIC able to convert light to chemical energy

PHOTOTROPH an organism that uses light for energy

PHYLOGENETIC pertaining to the evolutionary history of an organism

PILUS small protein projection on the bacterial cell involved in conjugation or adherence

PINOCYTOSIS the engulfment of liquid droplets by a cell

PLAQUE a clear area produced by the lytic action of viruses on an opaque lawn of bacteria or on a monolayer of tissue culture cells

PLAQUE (DENTAL) the structureless accumulation of bacteria, extracellular polysaccharides (e.g., glucans), and host proteins found on the surface of teeth

PLASMA the fluid portion of the blood containing elements necessary for clot formation

PLASMA CELL a fully differentiated B lymphocyte that produces antibodies

PLASMID an extrachromosomal piece of DNA

PLASMOLYSIS shrinkage of the cell caused by osmotic removal of water

PLASMOPTYSIS swelling or bursting of a cell caused by osmotic inflow of water

PLEOMORPHISM the state of having more than one form or shape

PMN polymorphonuclear leukocyte (neutrophil)

POCK a pit, spot, or pustule

POLYGENIC a messenger RNA coding for more than one gene

POLYMERIZATION formation of a polymer from monomeric molecules

POLYPEPTIDE a polymer made up of amino acids linked by peptide bonds

POLYSACCHARIDE a carbohydrate; for example, starch produced from the polymerization of many monosaccharides

POLYSOME (POLYRIBOSOME) several ribosomes bound to a single mRNA strand

POLYURIA frequent urination

PORINS channels in the cytoplasmic membrane for solute transport

PRECIPITIN an antibody that causes precipitation

PREDISPOSING conferring a tendency

PROCTITIS inflammation of the rectum

PRODROMAL pertaining to early manifestation of disease before specific symptoms appear

PROKARYOTIC characterized by lack of nuclear membrane and organelles (e.g., bacteria)

PROMOTER a site on DNA that initiates the transcription of an operon

PROPERDIN a bactericidal protein component of the blood

PROPHAGE state of a virus in which the viral genome is integrated into the host genome

PROPHYLAXIS protection, for example, against disease

PROTEOLYTIC able to break down proteins

PROTO-ONCOGENE a normal cellular gene capable of conversion to an oncogene

PROTOPLAST a viable bacterial cell without its cell wall

PROTOTROPH an organism capable of synthesizing

cell material from inorganic compounds

PROVIRUS virus integrated into host chromosome and transmitted from one generation to another

PRURITUS itching

PSITTACOSIS a bird disease, transmissible to humans

PSYCHROPHILE a microorganism that grows best at temperatures between 0° and 20°C

PUERPERAL related to the period after childbirth

PURINE an organic base found in nucleic acids

PURPURA dusky, blotchy hemorrhages in the skin or mucous membrane

PURULENT associated with the formation of pus

PUSTULE an elevated lesion filled with pus

PUTREFACTION decomposition of proteins resulting in foul odors

PYOCIN bacteriocin produced by *Pseudomonas aeruginosa*

PYOGENIC pus forming

PYRIMIDINE an organic base found in nucleic acids

PYROGEN an agent that induces fever

QUARANTINE to detain or isolate individuals because of suspicion of infection

QUELLUNG REACTION a test in which the bacterial capsule swells as a result of combining with specific antibody

REAGIN the nonprotective antibody found in the blood and produced in response to a number of different diseases; also, the antibody of immunoglobulin class IgE, involved in anaphylaxis and atopic allergies

RECALCITRANT resistant to change

RECOMBINATION in genetics, a process in which genetic information is exchanged

RECRUDESCENCE recurrence of symptoms after their abatement

REDUCTION gain of electrons or hydrogen

REGULATOR CELL a T helper or T suppressor cell that regulates an immune response

REGULATOR GENE a genetic unit on the chromosome that controls the synthesis of the repressor protein

REPLICATION a duplication process requiring a template

REPLICON any genetic unit capable of autonomous replication

REPRESSIBLE ENZYME an enzyme whose synthesis can be decreased by certain metabolites

REPRESSOR PROTEIN a protein whose function is to control the operator gene; the regulator gene controls the synthesis of the repressor protein

RESOLVING POWER the ability of a lens (or the eye) to distinguish two closely associated objects as distinct structures

RESPIRATION an oxidative process in which energy is released from foodstuffs

RETICULOENDOTHELIAL SYSTEM (RES) a network of phagocytic cells produced and residing in the bone marrow, spleen, lymph nodes, and liver of vertebrates

RHEUMATOID FACTOR a distinctive gamma globulin found in the serum of patients with rheumatoid arthritis

RHINITIS inflammation of the nose

RIBOSOME a ribonucleoprotein particle found in the cell cytoplasm and involved in protein synthesis

RINGWORM a common name for ring-shaped patches appearing anywhere on the body surface and caused by a group of fungi called dermatophytes; tinea

RUBELLA German measles

RUBEOLA measles

SACCHAROLYTIC capable of breaking down sugars

SALPINGITIS inflammation of the uterine (fallopian) tube

SANITIZE to reduce the number of bacteria to a nonhazardous level

SAPROBE an organism that derives its nourishment from dead or decaying material

SARCOMA a solid tumor growing from derivatives of embryonal mesoderm such as connective tissue, bone, muscle, and fat

SCROFULA tuberculosis of the lymph glands

SECRETORY IGA polymerized IgA with a secretory piece that appears on mucous membranes where it provides local immunity

SEPSIS a toxic condition resulting from the presence of microbes or microbial products in the body

SEPTICEMIA a systemic disease in which microorganisms multiply in the bloodstream

SEPTUM a dividing wall or partition

SEQUELA a morbid (abnormal) condition that develops as a consequence of a disease

SEROCONVERSION the induction of specific antibodies in the serum after their apparent absence from the serum

SEROLOGY the study of antigen-antibody reactions in vitro

SEROTYPE a taxonomic subdivision of microorganisms based on the kind of antigens present

SERUM the clear portion of the blood minus the factors necessary for clotting

SLOW VIRUS a virus that causes subacute or chronic disease with an incubation period that lasts several weeks to years before the onset of clinical symptoms

SMEAR a film of material such as a bacterial suspension spread on a glass slide

SPECIFICITY that property of a relationship that restricts an agent or a reactant to combine with or to affect a particular group, subject, substance, cell, or molecule

SPECTROPHOTOMETER an instrument used to measure the absorption of light in test liquids

SPHEROPLAST in bacteriology, a gram-negative cell in which the cell wall has been removed but some cell wall components remain

SPIKES surface projections of various lengths spaced at regular intervals on the virus envelope

SPIROCHETE a corkscrew-shaped bacterium

SPLENOMEGALY enlargement of the spleen

SPORANGIOSPORES asexual fungal spores produced in a sac called a sporangium

SPORANGIUM a structure that holds asexual spores

SPORE the resistant form of a bacterium derived from the vegetative cell; the reproductive cell of certain organisms

SPORICIDE an agent that destroys spores

SPOROGENESIS production of spores

STEATORRHEA excessive loss of fats in the feces

STERILIZATION the process that destroys or removes all living microorganisms

STOMATITIS inflammation of the oral mucosa

STREET VIRUS the virulent type of rabies virus isolated in nature from domestic or wild animals; see FIXED VIRUS

STREPTOLYSIN a hemolysin produced by streptococci

SUBACUTE between acute (short course) and chronic (persisting over a long period)

SUBCLINICAL without clinical manifestations of the disease

SUBSTRATE a specific compound acted on by an enzyme

SUPERINFECTION an infection superimposed on an already existing infection; for example, a bacterial infection on top of a viral infection

SUPPRESSOR T CELLS (T_S) a regulatory T lymphocyte that suppresses the responses of immune cells

SUPPURATIVE pus forming

SYNDROME a group of symptoms that characterize a disease

SYNERGISM a phenomenon in which the action of two components together is more than the sum of the two alone

SYNTHESIZE to build up a chemical compound from its individual parts

SYSTEMIC relating to the entire organism and not any individual organ

TAUTOMERISM existing in a state of equilibrium between two isomeric forms

TAXONOMY the orderly classification into distinct categories based on some suitable relationship between groups or individual organisms

T CELLS thymus-dependent lymphocytes involved in cell-mediated immunity

TEMPERATE PHAGE a nonvirulent bacteriophage

TEMPLATE a mold for the synthesis of new material

TERATOGENIC capable of inducing abnormal development and congenital malformations

THERMOLABILE sensitive to heat

THERMOPHILES microorganisms growing best at temperatures between 45° and 70°C

THROMBOCYTOPENIA a decreased number of blood platelets

THROMBOPHLEBITIS inflammation of a vein associated with thrombosis

THROMBOSIS formation of a clot within a blood vessel

THRUSH fungal infection involving the oral mucous membrane

TINCTURE an alcoholic solution of a particular substance, for example, tincture of iodine

TINEA a name applied to fungal infections of the skin; ringworm

TITER the concentration of animate or inanimate agents in a medium

TOXEMIA a condition in which toxins are in the blood

TOXOID a modified exotoxin that has been treated to destroy its toxicity and retain immunogenicity

TRACHOMA ocular disease caused by *Chlamydia trachomatis*

TRANSAMINATION a chemical reaction in which an amino group is transformed to a keto-acid

TRANSCRIPTION the formation of messenger RNA from a DNA template

TRANSDUCTION the transfer of bacterial genetic information from one cell to another by a virus

TRANSFERASE an enzyme that catalyzes the exchange of functional groups between compounds

TRANSFORMATION in genetics, a process in which the genetic constitution of a cell is altered through the uptake of free DNA from the environment

TRANSITION a mutation caused by substitution of one purine for another or one pyrimidine for another

TRANSPOSITION in genetics, the ability to be transferred to different sites on the nucleic acid molecule

TRANSPOSON an element of DNA containing insertion sequences as well as genetic information

TRIPLET CODE a code in which a set of three nucleotides specifies one amino acid

TRISMUS spasm of the masticatory muscles resulting in difficulty in opening the mouth (lockjaw)

TROPHOZOITE the active vegetative stage of a protozoon

TUBERCLE a nodule

TUBERCULOPROTEIN (TUBERCULIN) protein extract derived from *Mycobacterium tuberculosis*

TUMOR a new growth of tissue in which the multiplication of cells is uncontrolled; a swelling

ULCER a circumscribed area of inflammation characterized by necrosis and found in the epithelial lining

URTICARIA hives—a vascular reaction of the skin characterized by elevated patches (wheals)

VACCINE a suspension of organisms, usually killed or attenuated, used for immunization

VARICELLA chickenpox

VARIOLA smallpox

VASCULAR containing blood vessels

VECTOR a carrier of pathogenic microorganisms

VEGETATIVE concerned with the growing stage of a microorganism as opposed to the spore state

VENEREAL transmitted by sexual contact

VESICLE a blister; also a membranous unit derived from Golgi and involved in transport

VIREMIA presence of virus in the bloodstream

VIRION a complete virus particle consisting of a core of nucleic acid and a protein capsid

VIROGENE a viral gene incorporated in the host genome

VIROID an infectious subviral particle consisting of nucleic acid without a protein capsid

VIRULENCE the relative ability of an organism to cause disease

VOLUTIN see METACHROMATIC GRANULES

WHEAL a flat elevated area of skin caused by edema of underlying tissue

WILD TYPE in microorganisms the most frequently observed phenotype and often referred to as normal (as opposed to mutant) phenotype

YAW a lesion associated with the disease yaws

YAWS an infectious nonvenereal disease occurring in the tropics

YEAST a unicellular fungus

ZOONOSIS a disease of animals that can be transmitted to humans

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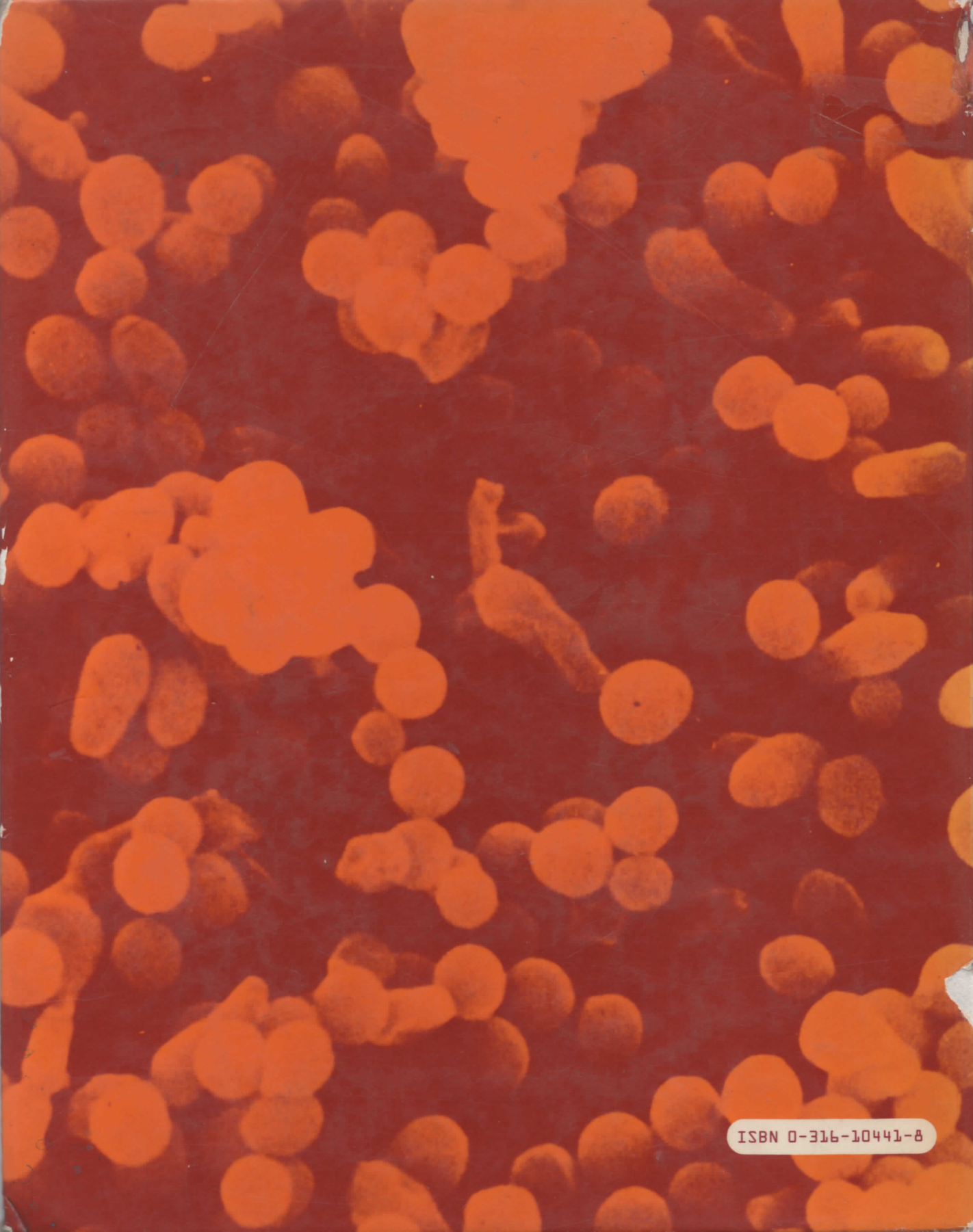

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